ELUCIDATING THE GENETIC BASIS OF BAST FIBRE PRODUCTION IN FLAX (*Linum usitatissimum* L.)

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By

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ABSTRACT

Flax is often considered a total utilization crop because of the potential to extract value from two distinct products - seeds and stem fibres. However, very little genetic information is available on flax fibre genetics in comparison to oil improvement studies. In order to gain a detailed understanding of genetic control of the fibre concentration and search for the possibilities of developing dual purpose flax lines using both seed oil and stem fibre, the following studies were initiated:

The first study evaluated the fibre and oil-related traits in a recombinant inbred population derived from a cross between a fibre flax variety Viking and an oilseed flax genotype E1747 over multiple locations under western Canadian field conditions. The study confirmed the presence of a significant genotype by environment interaction ($p < 0.01$) for fibre concentration indicating selection for this trait will be challenging. However, a lack of significant correlation between fibre and oilseed characteristics in field trials was encouraging and strengthened the hypothesis that breeding dual purpose flax types for western Canada is possible. The study also identified potential recombinant inbred lines (RILs) with enhanced fibre concentration as well as oil characteristics for use in future breeding endeavors.

The second study established an anatomical basis for further research into flax fibre improvement by studying differences between the stem anatomy of 14 diverse flax genotypes in the field and under controlled environments such as a growth chamber. The results from the study supported the use of controlled environments for the purpose of quick screening of high fibre containing genotypes, especially at the green capsule stage of plant growth. The results also indicated that it was possible to select high fibre oilseed flax lines based on anatomical markers such as average area of single fibre cells, total fibre area and fibre to stem area ratio.

In the third study, 17 simple sequence repeat (SSR) and 2 cleaved amplified polymorphic sequences (CAPS) molecular markers were used to assess the extent of genetic variability in the Viking × E1747 RIL population. CAPS markers LuFAD3A and LuFAD3B had the highest marker trait association ($p < 0.0001$) with linoleic and linolenic acid concentration. SSR markers such as CV8824, 5B6 and LU32 were found to be associated with plant height, oil concentration and protein concentration respectively using single marker analysis and step wise regression

analysis. The molecular study confirmed the importance of Viking × E1747 mapping population in identifying genes/ markers related to both fibre and oilseed related traits in flax.

In the fourth study, global transcript profiling using cDNA - based microarrays was performed to identify differentially expressed fibre related transcripts between Viking and E1747. The largest group of transcripts (7 %) found more abundant in Viking relative to E1747 fell under the functional group of cell wall development using gene ontology (GO) analysis. Transcripts such as callose synthases, expansins, cytochrome P450, fasciclin-like arabino galactan proteins and β-galactosidases were highly abundant in Viking relative to E1747. The transcripts more abundant in E1747 relative to Viking were UDP – glucose glucosyltransferase, auxin repressed protein, ubiquitin conjugating enzyme, peroxidases and lipid transfer proteins. Quantitative real time PCR results confirmed the suitability of the microarray platform to accurately discriminate transcript profiles between the two diverse flax types.

In conclusion, this research has provided a number of new insights into flax fibre genetics. This information lays the foundation for further genetic studies on flax bast fibres and will complement research on developing dual purpose flax varieties.

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LIST OF ABBREVIATIONS

ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
AFLP	Amplified Fragment Length Polymorphism
AGPs	Arabinogalactan Proteins
ARP	Auxin Repressed Protein
CAPS	Cleaved Amplified Polymorphic Sequence
CDC	Crop Development Centre
cM	Centi-Morgan
CV	Coefficient of variation
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
EMS	Ethylmethane Sulphonate
ESTs	Expressed Sequence Tags
FAME	Fatty Acid Methyl-Esters
G × E	Genotype × Environmental interaction
GA_3	Gibberellic Acids
GLC	Gas-Liquid chromatography
HCL	Hierarchical Clustering Analysis
IAA	Indole Acetic Acid
LSD	Least-Significant Difference
LTP	Lipid Transfer Protein
MAS	Marker Assisted Selection
MIDAS	Microarray Data Analysis Software
NDF	Neutral Detergent Fibre
NIR	Near Infrared Reflectance Spectroscopy
PAL	Phenylalanine Ammonia Lyase
PCR	Polymerase Chain Reaction
qRT-PCR	Qquantitative Real- Time PCR
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred Line
RNA	Ribonucleic Acid
SAHN	Sequential Agglomerative Hierarchical and Nested
SAM	Significance Analysis of Microarrays
SEM	Standard Error of the Means
SMA	Single Marker Analysis
SNPs	Single Nucleotide Polymorphism
SSRs	Simple Sequence Repeats
SWR	Stepwise Regression Analysis
TILLING	Targeting Induced Local Lesions in Genomes
UPGMA	Unweighted Pair-Group Method Arithmatic Average method

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CHAPTER 1

Introduction

Flax has been cultivated since the beginnings of civilization, and throughout the ages people all over the world have celebrated its usefulness. Cultivated flax belongs to the genus *Linum*, species *usitatissimum* (meaning "most useful") in the family Linaceae. *L. usitatissimum,* is of two types: one is grown for the seed oil and the other for its stem fibre. Flax grown for its seed oil is commonly referred to as oilseed or linseed flax, whereas flax grown for its stem fibre is generally termed as fibre flax. From hereon, flax grown for oil will be referred to as "oilseed flax" and flax grown for fibre will be referred to as 'fibre flax'.

Canada produces about one fourth of the world's oilseed flax and is the largest exporter of flax seed. The average (2000-2009) oilseed flax production in western Canada was 785.3 thousand tonnes with Saskatchewan producing 582.6 thousand tonnes (Statistics Canada, 2010a, b). The other major oilseed flax growing countries include China, USA and India, which together account for 40 % of the world production (Figure 1.1).

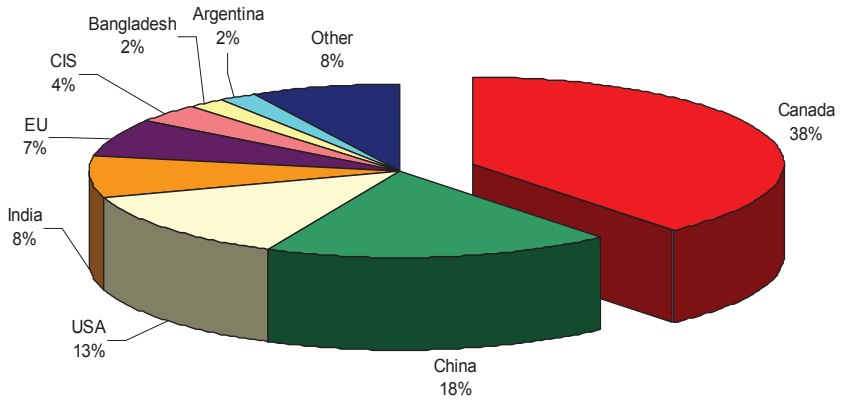


Figure 1.1. World production of flax seed in 2007-2008 (Agriculture and Agri-Food Canada, 2007).

Oilseed flax with its high omega-3 and omega-6 fatty acid content, ample fibre and cancer-fighting lignans is a unique functional food (Simopoulos, 2002). Besides the extraction of flaxseed oil, the seeds are also used in some food products, for example as an ingredient in

breads and bagels. Although the oil is edible, due to the presence of high linolenic fatty acid content (45–60%), it is used primarily for industrial purposes, such as the production of paints and oil-based coverings as well as the manufacturing of linoleum flooring. However, the introduction of low linolenic acid "solin" flax varieties increased the potential use of the crop in the edible oil industry and agriculture (Flax Council of Canada, 1998).

Flax also has a very long history of use as a natural source of fibre from the stem, especially in areas where cotton cannot be grown. In Europe, Russia and China, flax is grown for the production of fibres for linen textiles (Figure 1.2). In the European Union, France, Belgium, the Netherlands and the Czech Republic are the greatest producers of flax fibre for textile industries. Alternatively, fibres provide natural raw materials for biocomposites and pulp / paper. One of the potentially largest applications for flax fibres is in biocomposites, promoted in part for environmental concerns and as a replacement for glass fibre.

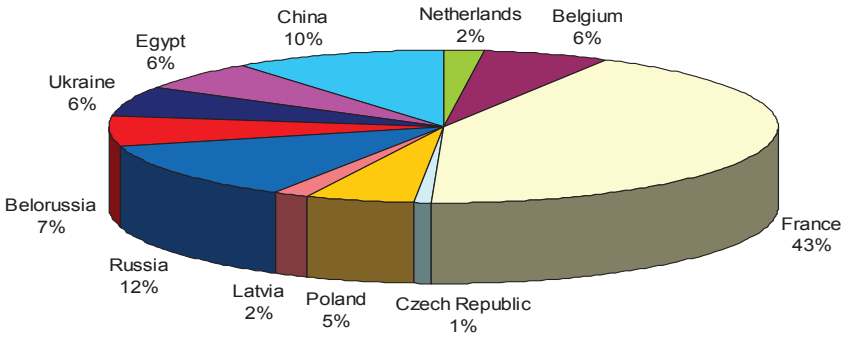


Figure 1.2. World fibre flax production, 2002-2003 (Frank, 2005).

Differences exist between fibre flax and oilseed flax due to plant morphology, varietal differences, environmental conditions as well as harvesting time and methods (Dimmock et al., 2005). Oilseed flax varieties are characterized by having a short stem, profuse branching and high seed / oil yield whereas the fibre flax varieties are characterized by a long slender stem with little branching and poor seed yield.

In Canada, the oilseed flax crop generates more than one million tonnes of flax straw, which constitutes a major environmental disposal problem due to the fibrous stems (Domier,

1996). Instead of struggling to incorporate flax straw back into the soil or have it chopped and spread, most of flax straw residue is burned in the field causing air pollution (Ulrich, 2006). Collection and removal of the straw residue is time-consuming and involves extra expense and time on the part of the farmer. If this straw could be efficiently processed, then it could produce a value-added natural fibre and also reduce environmental pollution caused by burning of millions of tonnes of straw.

New applications of flax fibre such as biocomposites and pulp and paper making do not require elongated or fine fibres which are needed by the textile industry (Kessler and Tubach, 1995) and this raises the question whether the same plant can be used for both purposes (viz., seed oil and stem fibre) thus making *Linum usitatissimum* a potential dual purpose crop. This would help the farmers to earn additional income, and eliminate the risks associated with burning of the flax straw.

Preliminary tests conducted in Saskatchewan have shown fluctuating amounts of fibre concentration between cultivars, but it is unclear which oilseed cultivars could consistently yield the highest straw fibre concentration when grown in western Canada (Burton, 2007). To make processing of the oilseed flax straw feasible and to expand the industry, a consistent high fibre concentration is required. In order to identify these superior fibre varieties, a fast and reliable screening method is needed for determining the stem fibre concentration. Near-Infrared spectroscopy (NIR) is an indirect method that allows this estimation using a small bundle of straw without the need of retting and actual fibre extraction (Barton et al., 2002).

A primary constraint to the development of dual-purpose varieties is the lack of knowledge about the genetic and molecular basis of fibre production in flax. Though plant breeders have been successful in developing low linolenic-acid edible oil flax for human consumption, very little information is available to understand the genetic architecture of genes responsible for fibre production in the growing stem of flax. It is also important to know the relationship between fibre and oil related traits and whether there is a possibility of combining both the traits into a single cultivar.

It is known that product quality and productivity can be improved by a better understanding of the material structure (Roland and Vian, 1991). In order to understand fibre development, it is very important to know in detail the ultrastructure of the fibre bearing stem tissues. Some work has been already done to anatomically characterize flax stems (Sankari, 2000a; His et al., 2001; Dijon, 2002) but these studies are not as numerous as those of other fibre crops such as cotton, ramie or jute. Molecular studies in flax are also relatively new as compared to other crops. Gene expression studies associated with xylem/ wood development have been already done in other species such as poplar, loblolly pine and Arabidopsis (Hertzberg et al., 2001; Whetten et al., 2001; Zhao et al., 2005) but studies on flax fibre have just been initiated (Roach and Deyholos, 2007; Fenart et al., 2010).

The purpose of this project was to provide fundamental knowledge about flax fibre genetics using conventional and molecular biology techniques. It will also be interesting to find markers of a physical, anatomical or molecular nature, which could help in easy and rapid screening of the high fibre lines. Furthermore, it will be rewarding to identify potential genotypes that could serve both oil as well as fibre markets and increase the economy of flax cultivation in Canada and rest of the world. Therefore, the objectives of the present study were:

1. Screening for flax dual purpose traits (seed oil and stem fibre) under western Canadian growing conditions.
2. Anatomical characterization of the flax stem with special reference to fibre production.
3. Molecular characterization of flax for dual purpose flax improvement.

CHAPTER 2

Review of literature

Origin and History

Flax (*Linum usitatissimum* L.) is an ancient crop cultivated for both its seed oil and stem fibre. The earliest cultivated flax species was *L. angustifolium*, a shorter perennial plant with smaller and narrower leaves than *L. usitatissimum* (Cullis, 2007). Because of great adaptability and diversity of flax, the origin of this crop is obscure (Lay and Dybing, 1989). However, it is believed that the crop was domesticated in the Near East about 10,000 years ago (Zohary and Hopf, 2000).

The first archeological discovery of flax was at Cayonu in southeastern Turkey and was dated to about 9000 years ago (Cullis, 2007). Flax usage has also been reported in Syria, Egypt, Rome and Greece as early as 8000 B.C. Flax came to North America approximately four hundred years ago, when it was brought to New France (Quebec City) in 1617 by Louis Hébert, the first farmer in Canada (Flax Council of Canada, 2006).

Taxonomy

Flax is an annual crop belonging to the family Linaceae that has been bred for two different needs: for its seed oil (oilseed), or straw fibre (fibre flax). Both crops were derived from the same species, *Linum usitatissimum*, through selection for different traits. The overall place of flax in the plant classification is as follows (Cullis, 2007):

Divison: Magnoliophyta

Order: Malphigiales

Family: Linaceae

Genus: *Linum*

Species: *Linum usitatissimum*

The Linaceae comprises 22 genera of which the genus *Linum* is the most well known. There are more than 200 species present in *Linum* and it can be further divided in five subsections: *Linum, Dasylimum, Syllinum, Cathartolinum* and *Linastrum* (Tutin et al., 1968). The subsection *Linum* contains the cultivated species *L. usitatissimum* and the ornamentals *L. grandiflorum* and *L. perenne*. However, the latter two species are of little economic importance. The wild progenitor of flax is thought to be *L. bienne* also known as pale flax (Fu et al., 2010). The number of chromosomes of the *Linum* species show a wide range varying from 2n = 16 to 2n = 72 (Fedorov, 1974). *L. usitatissimum* and its wild relatives contain 2n = 30 chromosomes (Muravenko et al., 2003).

Botany

Flax is a diploid (2n = 30) plant with high degree of self-pollination (Fedorov, 1974). Flower parts: petals, sepals and stamens all occur in units of five (Turner, 1987). The ovary has five carpels each of which is capable of developing two seeds (Schewe et al., 2011), for a maximum of ten seeds per boll (capsule). Petal color can range from dark to light blue, or from pale pink to white. Flowers in flax are perfect, regular, hypogynous, terminal in position (Beard and Comstock, 1980) and tetracyclic (Hayward, 1948).

In the field, flowers open shortly after sunrise, and the petals drop by the late afternoon (Freer, 1991). Some cross-pollination facilitated by insects occurs but at very low levels. The leaves of the flax plant are linear to lanceolate, sessile and without stipules. The leaf arrangement is variable: basal leaves are commonly arranged in alternate pairs, whereas those in the upper part of the stem are spirally arranged (Hayward, 1948). Generally the average life cycle of a flax plant in western Canada varies between 90 to 125 days (Flax Council of Canada, 2000). The first half of the cycle is known as the vegetative stage, and the latter half is made up of the flowering and maturation periods.

Flax Seeds

Flax seeds are flat, usually shiny and slippery due to the presence of a mucilage layer on the seed coat. The seeds are oval and pointed at the embryo axis end. Seed color varies among cultivars, and can range from light to dark brown or yellow. Flaxseed oil is mainly composed of five fatty acids, namely palmitic acid (16 carbon fatty acid with no double bonds), stearic acid

(18 carbon fatty acid with no double bonds), oleic acid (18 carbon fatty acid with a single double bond), linoleic acid (18 carbon fatty acid with two double bonds) and linolenic acid (18 carbon fatty acid with three double bonds). Ranges in content within flax seed of the fatty acids along with their omega designation are depicted below in Table 2.1.

Table 2.1. Fatty acid composition of flaxseeds (Murphy, 1994)

Fatty acid composition	**Palmitic acid (16:0)**	**Stearic acid (18:0)**	**Oleic acid (18:1)**	**Linoleic acid (18:2)**	**Linolenic acid (18:3)**
Omega designation			Omega- 9	Omega- 6	Omega -3
Range	4-7 %	2-8 %	12-38 %	5-27 %	26-65 %

The high concentration of alpha linolenic acid in flaxseed imparts a drying property to the seed oil upon exposure to air and has allowed the oil to be used for the manufacturing of coatings such as stains, paints, varnishes and other products like inks and linoleum (Green and Marshall, 1984). However, the edible property of the extracted oil is limited due to rancidity caused by its rapid oxidation upon exposure to air. In Canada, yellow oilseed varieties, usually assigned the name “solin” (Flax Council of Canada, 1995) are grown for edible - oil purposes and have less than 5 % alpha linolenic acid concentration in their seed.

Flaxseed processing plants also produce flaxseed meal which contains approximately 36 % crude protein and high amounts of omega-3 fatty acids (Morris, 2007). These meals can be incorporated into the rations of poultry and cattle to create products like omega -3 fatty acid enriched eggs, milk and cheese. Omega-3 fatty acids have shown potentially life-saving benefits in human health, such as preventing heart disease, as well as some types of cancer. Flaxseeds are also the richest source of lignans in food grains (Green, 1995). Lignans are possible anti-carcinogenic compounds, and are associated with reducing the risk of heart disease (Green, 1995; Berglund, 2002). Due to the health awareness, the use of flaxseed in breads, bagels and other baked goods has tripled in North America and is promoted as a functional food (Agriculture and Agri Food Canada, 2007).

However, the most important characters for oilseed flax breeding are high oil content and high oil quality. Oil content was found to be a quantitative trait and significantly influenced by environmental interaction (Comstock, 1966; Comstock et al., 1969). Oil concentration has been found to be positively correlated with oleic acid concentration and negatively correlated with protein concentration (Rahimi et al., 2011). As low linolenic acid content is one of the key factors for edible- oil purposes, it is important to understand the genetic inheritance of this fatty acid. Past research shows that there is a negligible correlation with oil concentration (Saeidi and Rowland, 1999) and that the linolenic acid level is controlled by two recessive genes present at independent loci (Rowland, 1991; Vrinten et al., 2005).

Flax fibres

Plant fibre in general represents a slender, elongated, threadlike object or structure. In botanical terms, fibres are one of the elongated, thick-walled cells that give strength and support to plant tissue (Esau, 1977; Gorshkova et al., 2003). Some of the world's most interesting and economically important fibre plants, used for cordage and textiles, come from sources like leaf fibres (sisal, yucca and abaca); seed fibres (cotton and coir); seed pod fibres (kapok and silk floss) and stem fibres of dicotyledonous species. The latter are often referred to as bast fibres (flax, jute, ramie, kenaf and hemp). Flax fibre plays a key role in the global fibre supply just after jute and coir (Figure. 2.1).

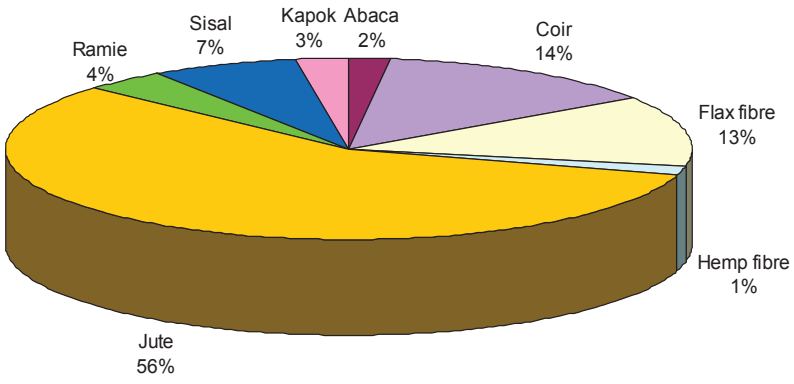


Figure 2.1. Global supplies of fibres. Source: FAOSTAT data, 2004.

Flax stem anatomy

Flax grows to a height of approximately 80-120 cm with fewer branches in fibre flax varieties and up to 40-60 cm high with profuse branching in oilseed varieties. The stem diameter of the flax plant ranges from 2-5 mm (Dijon, 2002). The flax stem consists of six consecutive layers. Starting from the centre of the stem, the core is filled by pith, which consists of thin walled parenchyma cells. Around the pith is the xylem (woody tissue), which conducts water and ionic solutes from the roots to the leaves. The xylem is separated from the phloem region by a ring of meristematic thin walled cells called the vascular cambium. These cambial cells are responsible for secondary growth of both phloem and xylem regions. Unlike xylem, phloem carries organic nutrients (photosynthates) from the leaves to all parts of the plants. The phloem contains the commercially important phloem (or bast) fibres. The fibre bundles consist of the single or ultimate fibre cells which form a continuous layer around the circumference of the stem (Esau, 1977). Outside this fibre layer is the cortex region containing pectins and chlorophyll. Finally, the outermost layer is the epidermis, which protects the stem from mechanical damage, water loss and infection (Figure 2.2).

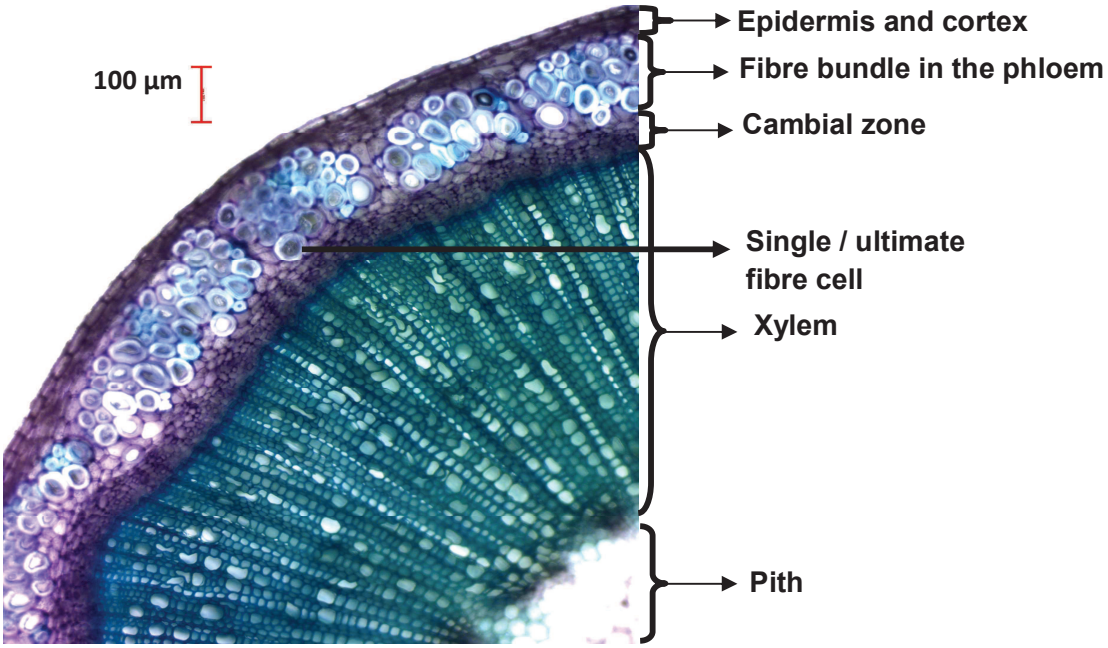


Figure 2.2. Cross-section of flax stem (variety Viking) at physiological maturity stage.

Individual (ultimate) fibre cells are made up of long multinucleate cells without septa, and originate from procambial cells in the protophloem (Akin, 2005; Morvan et al., 2003). These fibre cells are very long (average 2-5 cm) and have thick secondary walls (5-15 µm) (Morvan et al., 2003). Matured fibre bundles consist of 12-40 individual cells on average depending upon the flax variety studied. The slightly rounded fibre cells are usually pentagonal or hexagonal in shape (Dijon, 2002). Depending upon the reference, there are some variations in flax fibre characteristics (Table 2.2).

The fibre cell wall is made up of usually a number of layers: the primary cell wall (the first layer deposited during fibre development) and the secondary cell wall, which again is made up of two layers (inner and outer) and a tertiary cell wall (Haudek and Viti, 1978). A schematic diagram of the ultrastructure of single fibre cells is depicted in Figure 2.3.

Table 2.2. Characterization of flax fibre cells modified from Sankari (2000a).

Type	Number of bast fibre bundles in a stem	Number of individual fibres in a fibre bundle	Length of a single fibre (mm)	Diameter of a single fibre (µm)
Fibre flax	20-50[1] 30[2] 15-40[7]	10-30[1] 10-40[2] 12-40[7]	20-40 [1,5] 27[2] 25-35[4]	12-25[3] 23[2] 17-24[8]
Oilseed			3-50[6] 8-30[7]	 26-38[8]

[1]Dambroth and Seehuber (1988), [2]Berger (1969), [3]Hayward (1948), [4]Herzog (1989), [5]Fölster and Michaeli (1993), [6]Easson and Molloy (1996), [7]van Dam et al. (1994), [8]Sankari (2000a)

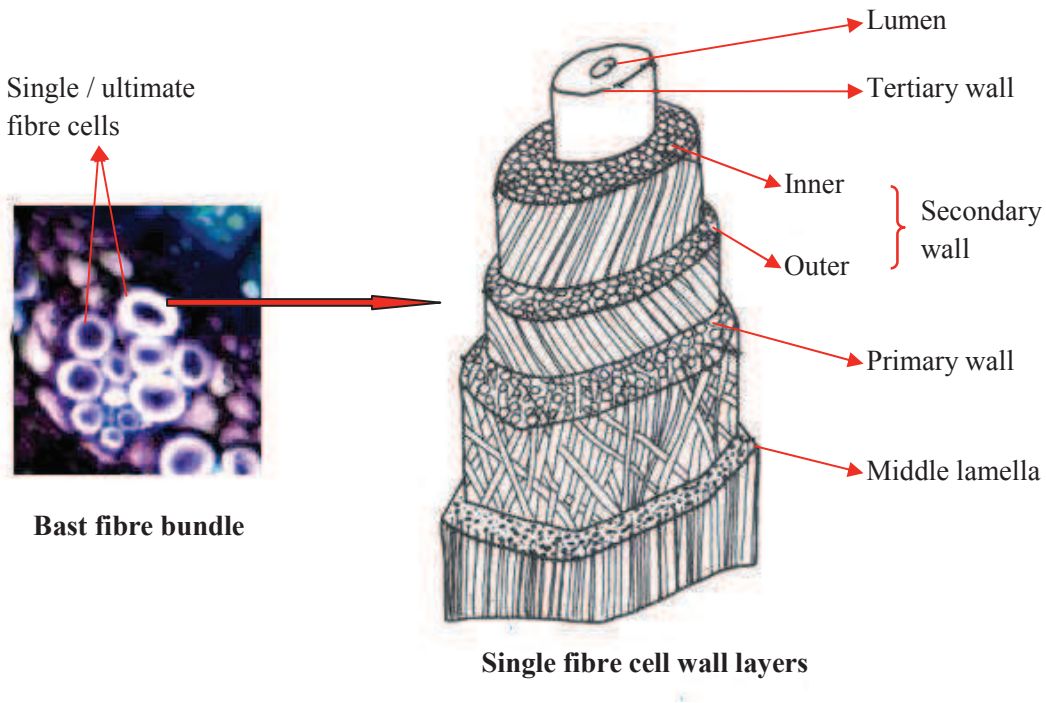


Figure 2.3. Structure of the bast fibre bundle and fibre cell wall layers of flax (modified from Haudek and Viti, 1978; Kymäläinen, 2004).

Flax fibre development

In general, plant fibre morphogenesis can be divided into four overlapping developmental stages: fibre initiation, primary cell wall synthesis (fibre expansion and elongation), secondary cell wall synthesis and maturation (Wilkins and Jernstedt, 1999). A flax fibre cell consists of a cell wall and a protoplast, the latter being the living part of the cell. The cell wall provides rigidity to the cell and prevents it from bursting in its hypotonic environment and allows the protoplast to generate turgor. Turgor is the key force in cell expansion and elongation and also provides rigidity to the unlignified cells (Dijon, 2002).

The developing stem of flax contains a specific region known as the "snap point", where the fibre enriched bast tissues considerably change their mechanical properties. The snap point was found to be present during a restricted period of plant development (the fast growth phase) and to disappear when stem growth is completed. The fast growth stage is the period when maximum length of all bast fibres in a mature plant is fixed and after which there is no change in fibre length. The snap point was shown to be the stem location, above which the fibre cells

elongate. Below this region however, no more elongation occurs, but intensive secondary cell wall thickening of the fibre cells takes place (Gorshkova et al., 2004).

The fibre cell elongation is related to that of the internode, which contains the fibre bundle and cell elongation is maximum during the fast plant growth phase (Morvan et al., 2003). The fibre elongation mechanism is not totally clear and is thought to undergo coordinated growth during early developmental stages, followed by intrusive (sliding) growth at later stages (Ageeva et al., 2005), but some authors also suggest an active surface type of growth, as observed in cotton (Esau, 1977).

The formation of secondary walls in fibre cells occurs centripetally, the first thickenings being observed immediately below the snap point region (Gorshkova et al., 1996). The first deposition coincides with the initiation of cambial activity (Roland et al., 1995). Studies suggest secondary wall formation of both fibre and xylem tissues depend on mineral nutrition, especially on the calcium to sodium ratio (Ripol et al., 1993), water supply and light intensity (Milthorpe, 1945). In flax, bast fibre maturation within stems, as determined by an increase in secondary cell wall diameter, occurs between the initiation of flowering and capsule maturation (Morvan et al., 2003).

Plant growth regulators play an important role in development of vascular tissues (Fukuda, 2004). Early physiological studies have established that fibre cell differentiation is induced along the paths of auxin polar flow, and requires three plant hormones, auxins, gibberellins, and cytokinins (Aloni, 1987). In jute and hemp plants treated with gibberellic acids (GA_3), fibres were four-fold longer than untreated plants (Moore and Clark, 1995). In flax, spray application of GA_3 stimulated stem elongation, stem expansion and number of bast fibres (McKenzie and Deyholos. 2011). The study also indicated that combination of GA_3 with indole acetic acid (IAA) increased the thickness of secondary fibre cell walls by two-fold. Another plant hormone, ethylene, has been shown to influence fibre elongation in cotton using ovule culture (Shi et al., 2006; Qin et al., 2007a), suggesting the importance of hormonal regulation in fibre development.

Flax fibre biochemistry

The major biochemical components of flax fibre cell walls have been identified as cellulose, hemicellulose, pectin, lignin, structural proteins and waxes. Chemical compositions of the flax stem relative to other natural fibres are shown in Table 2.3.

Table 2.3. Chemical composition of fibre from different plants (Olesen and Plackett, 1999).

Fibre	Cellulose	Hemi-cellulose	Lignin	Silica	Pectin	Ash
Cereal straw (Wheat)	40-45	20-31	15-20	3-7	8	2-5
Bast fibres						
• **Flax**	68-85	10-17	3-5		5-10	1-2
• **Jute**	70-75	12-15	10-15		1	-
Cotton	89-99	3-6	-		-	-
Deciduous wood	38-45	24-39	22-28			0.5
Coniferous wood	39-45	30-33	26-34		-	0.5

Cellulose is one of the major components of the flax fibre cell wall and is a linear polymer of β-D-glucose connected by 1,4-glucosidic linkages. The cellulose microfibrils form the scaffold of all cell walls, and are held together by cross linking glycans (Carpita et al., 2001). Cellulose is important in the life of plants as it provides necessary strength to resist the turgor pressure in plant cells. In addition, cellulose has a distinct role in maintaining the size, shape and overall structural integrity of the plants. As a result, most fibres are very strong, stable and resist degradation. The plasma membrane is the site of synthesis and assembly of cellulose microfibrils. The synthesis of cellulose requires several enzymes such as cellulose synthases that use UDP-glucose as the substrate. UDP-glucose is thus the main precursor of cellulose formation (Saxena and Brown, 2005).

Hemicelluloses are present in the middle lamellae as well as primary and secondary cell wall joining with cellulose and lignin (Focher, 1992). In contrast to cellulose microfibrils, hemicelluloses are flexible polysaccharides. These forms act as tethers that bind together into a network or as springs to keep microfibrils from getting too close together. Hemicelluloses are very hydrophilic (i.e., containing many sites to which water can readily bond). Hemicelluloses

mostly include xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan (Andeme-Onzighi et al., 2000).

Pectins are acidic and irregular polysaccharides that are present in the middle lamella and primary cell walls of flax (Focher, 1992). Pectins contribute to mechanical properties of the cell wall, influence cell adhesion, and are very easy to dissolve (Teleman, 2003). They are a family of complex polysaccharides that contain 1, 4-linked α-D-galacturonic acid. To date, three classes of pectic polysaccharides have been characterized: homogalacturonans, rhamnogalacturonans, and substituted galacturonans (Carpita et al., 2001). In contrast to cellulose, hemicellulosic and pectic polysaccharides are synthesized in the Golgi apparatus (Scheible and Pauly, 2004). UDP-D-glucuronate synthesis is hypothesized to be the rate limiting step for both hemicellulose and pectin formation (Hertzberg et al., 2001).

Lignin is a latin word for wood and it gives rigidity to a plant. Lignin inhibits the breakdown of pectins, the "cementing" material between ultimate fibres. The process of lignification is a complicated one and it has been reported that the concentration and structure of lignin in flax changes during the plant's growth cycle (Lewis and Yamamoto, 1990; Day et al., 2005a). Lignification has three steps: (a) biosynthesis of the monolignols (e.g. p-coumaryl, coniferyl, and sinapyl alcohols), (b) transport and secretion of monolignols, and (c) polymerization of the monolignols by dehydrogenation (Minorsky, 2002). The three major monolignols p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol give rise to the so-called H-units (p-hydroxyphenyl), G-units (guaiacyl) and S-units (syringyl), of the lignin polymer, respectively (Huis et al., 2012). The relative proportion of the different monolignols varies between different plant groups, species and tissues. In flax, the phloem fibres lignin mainly consists of guaiacyl (G) units in contrast to the mixed guaiacyl-syringyl (G-S) lignin type occurring in xylem fibres (Day et al., 2005a). Several enzymes such as phenylalanine ammonia lyases (PAL), peroxidases and laccases are involved at different stages of formation (Whetten et al., 2001). The monolignol glucosides are thought to be transported from the vacuole to the cell wall at the appropriate time during secondary cell wall deposition.

Callose is widely distributed within plant species, but in much lower quantities than cellulose. Callose is composed of a triple helix of a linear homopolymer of β-1,3-linked glucosyl residues with occasional β-1,6-linked branches (Stone and Clarke, 1992). The different linkage

between the glucosyl residues gives callose quite different physico-chemical properties when compared to cellulose. Callose is deposited at the cell plate of dividing cells, at plasmodesmata and sieve plates and in pollen mother cells and tubes. In higher plants, callose deposition is also induced in the plasma membrane under certain physiological conditions like in mechanical wounding, chemical treatment and pathogen attack. The deposition of callose at the cell plate also precedes the synthesis of cellulose, which is the major component of the fibre cell wall (Ruan et al., 2004).

Fats and waxes are found in minute amounts in flax stems and are located in the cuticle and their function is to protect the plant from drying (Haudek and Viti, 1978). Other cell wall components include structural glycoproteins, arabinogalactan proteins (AGPs), and enzymes. The main structural glycoprotein in the cell wall of dicots is extensin, which is present in widely varying quantities, making up 1-10% of the wall (Fry, 1988). Extensins provides rigidity and strength to the primary cell wall by cross-linking with one another (Brady et al., 1996) or with other cell wall components, like pectins. Expansins another group of glycoproteins, are the primary agents inducing cell wall expansion by breaking hydrogen bonds between cellulose and hemicellulose (Cosgrove, 1999; Snegireva et al., 2010).

AGPs are a family of structurally complex water-soluble proteoglycans (Gaspar et al., 2001), which are found in the apoplastic fluid of many plant species and have a role in cell expansion and cell differentiation. Cell walls contain numerous enzymes, including those involved in cell wall metabolism (β-galactosidase, glucanases, xylanases, methyl and acetyl esterases, transglucosidases) and enzymes that may generate cross-links between wall components (e.g. peroxidases).

Flax fibre breeding and genetics

The structure, composition and arrangement of the fibre bundles determine fibre quality and yield, and is dependent on genotype and husbandry (van Sumere, 1992). Dimmock et al. (2005) suggested that environment played a huge role in the amount of fibre laid down in flax. Comparison studies of fibre yield and fibre quality across genotypes, locations and years makes it difficult because of the dependence on stage of development and analysis method (comparative data of flax summarized in Table 2.4).

Table 2.4. Reported biomass, fibre and seed yields for fibre and oilseed flax in experimental studies (Weightman and Kindred, 2005).

Reference	Genotype	Place (*)	Total DM yield (t/ha)	Seed yield (t/ha)	Straw yield (t/ha)	Fibre yield (t/ha)	Fibre (%)	Seed harvest index
Easson, 1989	Fibre	NL			3.7- 6.0	1.1- 1.8	26.5-30.9	
Aufhammer et al., 2000	Oilseed	DE	6.8-7.4	2.0-2.3				0.28- 0.32
Easson & Molloy, 2000	Fibre	NL	11	1.2	8	2	25	
	Oilseed	NL	8	2.2	5.2	0.7	16	
Dimmock et al., 2005	Fibre flax, 30 varieties	UK	6.3-11.1	0.72	3.39-5.11	0.8-3.7	39-59	
Couture et al., 2002	Fibre	CA	3.0-4.4					
Sankari, 2000b,c	Oilseed	FL		1.0-1.9	0.9-2.1			
Hassan & Leitch, 2001	Oilseed	UK	10-11					
Marshall et al., 1989	Fibre	CA	5-14	0.87-1.32				18.2-57.1

*, NL, Netherlands; DE, Germany; CA, Canada; FL, Finland; UK, United Kingdom

With regard to environmental conditions, temperature has a large impact on fibre yield via its effect on the rate of development and period of growth (Casa et al., 1999). Ideal weather conditions for growing fibre flax would consist of adequate moisture during the stem elongation stages, and long sunny days during flowering and ripening (Sheppard and Bates, 1988). Extreme temperatures such as frost or drought can severely harm flax fibre development (Foulk et al., 2004).

Other factors reported to affect the fibre content were seeding date (Dimmock et al., 2005; Burton, 2007), fertilization (Dimmock et al., 2005), plant density (Sankari, 2000a), precipitation (Menge-Hartmann and Höppner, 1995), time of harvest (Cromack 1998), stem part from where the fibre content was determined (Hoffmann 1961), the process by which fibres were

separated from the stem (retting) (Keijzer and Metz, 1992; Easson and Long, 1992a), stem length (Kaul et al., 1994) and stem diameter (Kaul et al., 1994; Turner 1987).

Fibre content ranged from 17-23 % in "non-commercial" varieties and 25-35 % in commercial varieties of fibre flax (Booth et al., 2004). Fibre content in the fibre flax straw was found to increase from flowering to maturity from 17 % to 25 %, while it remained constant at approximately 16 % in oilseed flax varieties (Easson and Molloy, 2000).

Kaul et al. (1994) reported a positive correlation between plant height and fibre yield in fibre flax wheras Sankari (2000a) suggested that even though the fibre flax cultivars produced significantly taller plants than oilseed types, they did not produce significantly higher stem or fibre yield due to low plant density. Kaul et al. (1994) found a negligible negative correlation between fibre content and oil yield characters, suggesting that both fibre content and oil content could be improved simultaneously in a single genotype. A moderate and positive correlation was also found in a study between fibre weight and total seed weight, and a completely independent inheritance in 100 seed weight (Foster et al., 1998), supporting earlier results that fibre content could be improved without adversely effecting oil traits.

Commercial breeding of flax started at the end of the 19th century. Fibre yield breeding can be divided into two components, straw yield and fibre content (Popescu et al., 1998). The low heritability found for fibre yield suggests a considerable environmental influence (Fouilloux, 1989; Couture et al., 2002; Mourad and Abo-Kaied, 2003; Dimmock et al., 2005). Fibre concentration (ratio of fibre weight on the stem) is more heritable and easier to determine. Furthermore, it is known that both additive and dominant effects of genes are involved in the heredity of fibre content and both effects are influenced by environmental conditions (Popescu et al., 1998). However, it is still uncertain how many genes are involved in the heredity of fibre concentration and straw yield. Several plant breeding methods or selection techniques are available with respect to flax breeding, but the pedigree method is the most common one used (Salas and Friedt, 1995).

Flax molecular markers

Molecular markers are powerful tools for gaining insight into the inheritance of complex quantitative characters. Molecular markers are being used for both the 'dissection' of complex agronomic traits as well as for the development of marker-assisted breeding strategies. The first molecular markers used to study the genetic diversity of flax were isozymes (Månsby et al., 2000).

Flax genetic diversity was assessed using phenotypic measurements (Diederichsen and Hammer, 1995; Diederichsen et al., 2002), using RAPD markers (Fu et al., 2003; Fu, 2005) and amplified fragment length polymorphism (AFLP) markers (van Treuren et al., 2001). Chen et al. (1998) combined RAPDs with inter-simple sequence repeat (ISSR) to identify microspore-derived plants in anther culture of flax. ISSR-based clustering of cultivated flax germplasm was also statistically correlated to thousand seed mass by Wiesner and Weisnerová (2004). However, none of the data has been placed onto a more general linkage map. Linkage mapping and quantitative trait loci (QTL) mapping using AFLP, RAPD and ISSR markers have been done for fatty acid pattern in flax seed oil (Klocke, 2000). QTL mapping of resistance and quality traits including fibre and oil traits in flax (*L. usitatissimum*) was conducted by Vroman (2006) based on AFLP markers. Nowadays, simple sequence repeats (SSRs) are becoming markers of choice due to their high level of reliability, co-dominant inheritance and genome coverage (Parida et al., 2009; Kalia et al., 2011). However, the development of SSRs in cultivated flax genetic research is still in its infancy.

Two approaches have been initiated, one using genomic SSRs and the other based on expressed sequence tag SSRs (EST-SSRs) (Roose-Amsaleg et al., 2006; Cloutier et al., 2009, 2010; Deng et al., 2010; Soto-Cerda et al., 2011a). Although this molecular marker approach represents an advance in molecular tools for flax breeding, they are still limited in number in order to analyze comprehensively the flax genome and efficiently use marker assisted selection (MAS) for improving stem fibre.

Flax fibre microarray

A microarray is a tool for analyzing gene expression on a small membrane or glass slide containing samples of DNA from many genes arranged in a regular pattern (NCBI, 2007). DNA microarrays are small, solid supports such as glass microscope slides, onto which the sequences from thousands of different genes are immobilized, or attached, at fixed locations. Microarrays exploit the DNA-RNA base pairing complementarity principle. For DNA, adenine (A) bases complement thymine (T) bases and vice versa; guanine (G) bases complement cytosine (C) bases and vice versa. With RNA, it is the same except that adenine (A) bases complements uracil (U) bases instead of thymine (T) bases. Since there is only one complementary base for each of the bases found in DNA and in RNA, one can reconstruct a complementary strand for any single strand.

The basic steps of microarray experiments includes extraction of mRNA from two different cell types, reverse transcription to form cDNA followed by labeling each with a separate florescent dye. The next step is to hybridize equal quantities of cDNA to the DNA chip and then washing off any unbound cDNA. The final step of a microarray experiment is detecting bound cDNA using laser technology, storing the resulting data in a computer file and later analyzing the ratio of fluorescent dye intensities at each spot in order to estimate the gene expression levels of the two cell types. Thus microarrays provide a high throughput tool for studying the temporal expression patterns of thousands of genes in a single experiment quickly and efficiently.

Microarray studies on cotton fibre development, especially the early stages of fibre initial formation in the growing cotton ovule (Ji et al., 2003; Taliercio and Boykin, 2007) have been reported. Studies on cotton fibre cell development showed complex processes involving many pathways, including many signal transduction, transcriptional regulation components and phytohormonal regulation (Lee et al., 2007). Auxin and gibberellins were shown to promote early stages of fibre initiation, whereas ethylene and brassinosteroids related genes were found to be up-regulated during the fibre elongation phase.

Global transcript profiling using cDNA or oligonucleotide-based microarrays to study mechanisms that regulate plant vascular development have been a longstanding interest to many

researchers. Hertzberg et al. (2001) used poplar cDNA arrays to profile changes in gene expression at various stages of secondary xylem differentiation. Oh et al. (2003), Koh et al. (2002) and Ko et al. (2004) used oligonucleotide arrays to identify genes expressed during secondary xylem growth in Arabidopsis. However, very few of the researchers on vascular biology have concentrated on phloem tissues, especially bast fibres. De Pauw et al. (2007) studied the transcript profiling of hemp phloem tissues and Roach and Deyholos (2007) investigating flax phloem tissues compared the gene expression in different locations along the height of the stem sections. Recently, the applicability of microarrays to detect differential gene expression was illustrated by comparing two diverse flax varieties with respect to disease resistance and fibre quality using different plant organs such as seed, leaves, stems and roots (Fenart et al., 2010).

Some of the interesting transcripts that were highly expressed in the above-mentioned flax fibre studies were those encoding cellulose synthases, fasciclin - like arabinogalactan proteins (AGPs), lignin pathway transcripts such as phenyl ammonia lyases, hemicelluloses and pectins such as xyloglucans and β-galactosidases, as well as lipid transfer proteins. Recently, the role of β-galactosidases in cell wall remodeling and secondary cell wall deposition was also confirmed using transgenic flax lines with reduced β-galactosidase activity (Roach et al., 2011).

Concept of dual purpose flax

Oilseed flax represents only 1 % of the world supply of plant oils, while flax fibre represents about 3 % of the world natural fibre production. Recently, the uses of flax fibres have been undergoing a considerable change. Public demand for natural products has increased enormously in the past 10 years and the fibre industry is replacing synthetic fibres with natural ones such as those from flax whenever possible (Smeder and Liljedahl, 1996). Unfortunately, the traditional linen textile industry suffers from regular cycles of boom and recession as linen goes in and out of fashion (Cooksley, 1996). However, new applications of flax fibre such as composites and pulp and paper production do not require the very long or fine fibres needed by the textile industry (Kessler and Tubach, 1995). It has been estimated that 25,000 tonnes of natural fibre like flax, hemp, jute and kenaf are being used in automotive industries alone with a prediction of even greater use in the future (McDougall et al., 1993; Karus, 1995). This potential

demand increases the possibility of using the flax plant for producing both fibre and seed, thus making *L. usitatissimum* a dual purpose crop.

Canada and USA export the majority of flaxseed used in the world. The straw residue from the crop degrades slowly due to the fibrous stem. Collection and removal of this straw is time-consuming and involves extra expense on the part of the farmer. There are three management practices used by farmers to deal with the straw namely; spreading, burning or baling. Flax residue when spread over the field unevenly often causes variable germination resulting from variable soil temperatures and soil moisture (Ulrich, 2006). Baling the straw to a processor for use in golf courses and horse stables is another option but the present market for such uses is very limited and usually not viable for farmers. Instead of struggling to incorporate flax straw into the soil, most of the residue is burned in the field (Ulrich, 2006). This creates air pollution, which is becoming an increasing concern with respect to human health and the environment. Burning of flax residue in the fields, also leaves fields vulnerable to wind and soil erosion. If this straw could be efficiently processed, then it could produce a value-added natural fibre and also reduce environmental pollution caused by burning of millions of tonnes of straw. A list of different products made from flax is found in Table 2.5.

Table 2.5. Uses of flax components (UK Flax and Hemp production, 2005).

Fibre					**Seed**
Textile	**Technical textile**	**Other industrial products**	**Paper**	**Building materials**	
Apparel Diapers Fabrics Handbags Working cloth Fine textile from cottonized fibre	Twine Rope Nets Canvas Bags Carpets Geotextile	Agro-fibre composite Compression-moulded parts Automotive interior parts	Printing paper Filter paper Newsprint Cardboard and packaging	Fibre board Insulation material Fibre glass substitute Cement block	Edible oil Oil paints Solvents Varnishs Chain-saw lubricants Printing inks Coatings

However, there are several limitations for breeding new dual purpose cultivars to meet both the markets of oil as well as fibre. A consistently high straw fibre concentration is required for the harvest of fibre from oilseed flax to be economically viable. Preliminary tests conducted in Saskatchewan have shown fluctuating amounts of fibre between cultivars, but it is unclear which oilseed cultivars could consistently yield the highest straw fibre concentration when grown in western Canada (Burton, 2007).

Opinions vary on the best way to approach the breeding of dual-purpose flax crops. The present oilseed and dual-purpose varieties have not been able to match flax varieties for fibre yield (Dimmock et al., 2005) and there may be physiological reasons for expecting some degree of compensation between seed and fibre components. Another problem with dual-purpose crops is the different rate of maturation of seed and fibre, with fibre typically reaching optimum maturity around three weeks before maximum seed yield is reached (Foster et al., 1997). By the attainment of seed maturity, the fibre can become coarse and lignified, which is of little or no value for linen. Some attempts have been made to address the synchronisation of stem and seed maturity through breeding (Keijzer and Metz, 1992; Foster et al., 1997). However, with the present usage in biocomposites, time of harvesting and lignification may not be a problem and can even be desirable for some markets.

Easson and Molloy (2000) previously pointed out that oilseed flax can yield only about 1/3 the fibre yield of fibre flax, whereas fibre flax can yield 60% of the seed yield of oilseed varieties, so selection for dual purpose varieties should be based on fibre flax varieties. However, others have recognized that the seed component has been the most important economically, and considering that fibre length and fineness is less important for many modern applications, opted for oilseed, or oilseed x fibre flax as parents for dual varieties (Foster et al., 1997; 1998; 2000; Sankari, 2000b, c). Foster et al. (1997) also found less variation for the important traits in fibre flax as compared to oilseed flax indicating better chances of improvement in oilseed type cultivars. They considered a hybrid approach for dual-purpose varieties but found insufficient heterosis for producing hybrid seed on a commercial scale. Therefore, they suggested development of recombinant inbred lines (RIL) produced from crossing oilseed, or oilseed x fibre flax as parents for breeding dual purpose varieties. However, reports to date to find viable

dual-purpose varieties have met with only limited success (Foster et al., 2000; Dimmock et al., 2005).

Spectroscopic screening methods

Previously, plant tissues had to be "retted" (a process separating the fibres from the rest of the stem) in order to estimate the fibre content, which is time-consuming and labor-intensive. Near infrared reflectance spectroscopy (NIR) has a wide range of applications in agricultural research starting from estimation of moisture, starch, protein, color intensity and oil content measurements in seeds and other agricultural produce (Sohn et al., 2004a, b; Nandy et al., 2007). Recently, with the advances in software technology, straw fibre content in flax can now be easily estimated using NIR (Barton et al., 2002). NIR analysis facilitates selective breeding for straw fibre concentration since straw fibre can now be measured relatively quickly and in a non-destructive manner.

NIR works on the principal of Beer's Law, which states that "absorption is proportional to concentration". The NIR instrument measures the number of photons which undergo the absorption process for a particular wavelength (Analytical Spectral Devices, Inc., 2006). The number of photons absorbed is proportional to the amount of a specific type of molecule present in the sample. A group of plant samples with a maximum range of variability is previously scanned and compiled using NIR and then the same group of samples is tested using standard analytical methods to measure the trait of interest (Burton, 2007). Then both the independent and NIR spectral data are compared using chemometrics software. A mathematical model is then created that best describes the relationship between particular spectral features and the trait of interest. This entire procedure is called a calibration. Therefore, whenever a new sample is scanned, the calibration interprets the information coming from the NIR instrument, and gives a prediction of the trait of interest in that plant sample.

The Crop Development Centre (CDC) at the University of Saskatchewan, Canada, is attempting to increase the straw fibre concentration in Canadian oilseed flax using the NIR fibre estimation method. Multi-location trials have been conducted throughout Saskatchewan to investigate the fibre concentration of different oilseed and fibre flax cultivars (Ulrich, 2006). The average fibre concentration estimated by NIR for fibre flax variety Hermes was 20.2 % and for

oilseed varieties CDC Mons, Solin cultivar SP 2090, AC Hanley and CDC Bethune were 11.8%, 15.1 %, 14.5 %, 14.0 % and 12.7 %, respectively (Burton, 2007).

Previously, Himmelsbach and Akin (1998) had indicated the use of Near – Infrared Fourier- Transform Raman Spectroscopy to detect major chemical components in flax stems indicating its potential use in simple, rapid and non-invasive method for flax fibre quality assessment. Toonen et al. (2004) attempted to perform NIR calibrations to measure acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) in core and bast fractions of hemp but met with very limited success. This limitation indicated the need for further research into evaluating fibre quality using spectroscopic methods.

CHAPTER 3

Screening for flax (*Linum usitatissimum* L.) dual purpose traits (seed oil and stem fibre) under western Canadian growing conditions.

Abstract

The genetic control of stem fibre and seed oil related traits in flax, *Linum usitatissimum* L. was investigated, in order to look into the possibility of breeding dual purpose varieties. Traits under study included fibre concentration, plant height, oil concentration, protein concentration and fatty acid profiles. To establish an appropriate study system, a 95 entry recombinant inbred line (RIL) population derived from a cross between a fibre flax (Viking) and an oilseed flax (E1747- an EMS mutant) genotype were evaluated at one location in 2005 and over three locations in 2006 under western Canadian field conditions. Significant variability was found between genotypes for all the traits and at all the locations. The study confirmed the presence of a significant genotype by environment interaction ($p < 0.01$) for fibre concentration indicating that selection for this trait will be challenging. However, a lack of significant correlation between fibre and oilseed characteristics was encouraging and strengthened the hypothesis that breeding for dual purpose flax types would be possible. Individual RILs with enhanced fibre concentration as well as oil characteristics were identified for use in future breeding endeavours.

Introduction

Linum usitatissimum is better known as either an oilseed (linseed) or a fibre crop (fibre flax). Although these types both belong to the same species, due to selective breeding, they now possess very distinct morphologies and are grown commercially as different crops. Virtually all the flax grown in North America is for seed-oil (oilseed type). Saskatchewan, North Dakota and Manitoba are primary regions that produce oilseed flax followed by Alberta and South Dakota. The main fibre flax growing countries are found within the Europian Union. However, France, China and Belgium are the major exporters of linen and finer flax fibres to the rest of the world.

In Canada, the straw residue from the oilseed flax degrades slowly in the field due to bast fibres in the stem. Collection and removal of the straw is time-consuming and involves extra expense on the part of the farmer. Instead of struggling to incorporate flax straw into the soil,

most of the residue is burned in the field (Ulrich, 2006) causing air pollution, health and environmental hazards. Burning of flax residue in the fields, also leaves fields vulnerable to wind and soil erosion. If this straw could be efficiently processed, then it could be used to produce a value-added natural fibre while at the sametime reduce environmental pollution caused by burning of millions of tonnes of straw.

Recently, the uses of flax fibres have been undergoing considerable change. Public demand for natural products has increased enormously in the past 10 years and the fibre industry is replacing synthetic fibres with natural fibres such as those from flax whenever possible (Smeder and Liljedahl, 1996). However, the traditional linen textile industry lacks stability as linen goes in and out of fashion (Cooksley, 1996). New applications of flax fibre such as bio-composites for automobile parts, pulp and paper making do not require the very long fine fibres, which are needed by the textile industry (Kessler and Tubach, 1995). This flexibility increases the possibility of using the same plant for producing both fibre and seed, making *L. usitatissimum* a potential dual purpose crop.

However, there are several limitations for breeding new dual purpose cultivars to meet both the markets of oil as well as fibre. A consistently high straw fibre concentration is required for the harvest of fibre from oilseed flax to be economically viable. Preliminary tests conducted in Saskatchewan have shown fluctuating concentrations of fibre among cultivars, but it is unclear which oilseed cultivars can consistently produce good straw fibre concentration when grown under western Canadian growing conditions (Burton, 2007).

Several studies on manipulating the flax seed oil properties, including the fatty acid profile, have been undertaken for use in diverse industrial and human consumption purposes (Saeidi and Rowland, 1997; Ntiamoah et al., 1995; Rowland and Bhatty, 1990). Unfortunately, information about the genetic architecture of fibre concentration is not clear. In order to gain a detailed understanding of the genetic control of fibre concentration and to look at the possibility of selecting dual purpose varieties, recombinant inbred lines (RIL) derived from a cross between a fibre flax and an oilseed parent were evaluated for both fibre as well as oil related traits over multiple locations. The present investigation had three major objectives: first, to serve as a preliminary investigation into the genetic control and the extent of relationship between commercially important traits of flax for both stem fibre and seed oil; second, to characterize a

RIL population derived from diverse genotypes, Viking (fibre flax) and E1747 (oilseed flax); and third, to identify potential dual purpose genotypes with improved fibre content as well as good seed oil characteristics.

Materials and Methods

Plant materials and field description

A RIL population consisting of 95 lines was developed by crossing a fibre flax genotype (Viking; PGRC Accession # CN 18987) with a low linolenic acid oilseed genotype (E1747). The RIL population ($F_{2:6}$) was developed using the single seed descent method. The performance of 95 RILs along with the two parent (Viking and E1747) and three checks (Hermes, Flanders and Somme) were evaluated.Viking and Hermes are European fibre cultivars, expected to have high straw fibre concentration, tall plant height, low seed oil concentration and high linolenic acid concentration. E1747 is a mutant line of the oilseed cultivar McGregor (Rowland, 1991) with < 5 % linolenic acid concentration making it suitable for commercial edible-oil markets. Flanders (Rowland et al., 1990a) and Somme (Rowland et al., 1990b) were both registered by the Crop Development Centre (CDC) as oilseed flax cultivars in Canada. They were classified as having small to medium seed size with good oil content and high linolenic acid concentration, medium to late maturity with very good resistance to lodging.

Field trials were conducted at the University of Saskatchewan, Kernen Crop Research Farm, Saskatoon, Saskatchewan in 2005 and at three locations in Saskatchewan (Saskatoon, Floral and Melfort) in 2006. The Kernen Crop Research Farm (52° 10.200' N**,** 106° 43.200' W, elevation 503.80 m) has a Dark Brown Chernozem (Typic boroll) Sutherland clay soil type. The soil type at Floral (Saskatchewan Pulse Growers' land) (52° 04.00' N, 106° 28.00' W elevation 503.80 m) is a Elstow Association with a clay- clay loam texture whereas the soil type in Melfort, Agriculture and Agri-food Canada Farm (52° 49.200' N **,** 104° 36.0' W, elevation 480.10 m), is of Orthic Black Chernozem and silty clay soil texture. Temperature and rainfall data during the crop growing seasons for both the years and locations are shown in Table 3.1.

The seeding rate for all field trials was 30 kg / ha with an average plant density of 400 plants / m^2. Border plots of Vimy were seeded at the edges of each experiment. Field trials at all locations were conducted using a randomized complete block design. The plot size was 6 rows

at 30 cm spacing between rows and 3.7 metres long.The sowing date in 2005 at the Kernen Crop Research Farm was May 20th. The sowing dates for the 2006 multi-location trials were May 19th at Kernen and Floral; and May 25th at Melfort.

Good weed control during early crop growth was achieved by applying the selective herbicides Buctril M® (Bromoxynil + MCPA), Centurion and Basagran. Plots were also hand weeded as needed throughout the growing season. No fertilizer was applied at any site and the crop was completely rain-fed. Reglone® (Diquat) was the desiccant used for all the field trials in order to achieve uniform maturity of the plants.

Traits under study included stem fibre concentration, plant height at maturity, total seed oil concentration, total seed protein concentration and seed fatty acid composition. Fatty acids measured in the study were stearic, palmitic, oleic, linoleic and linolenic. Three plant height measurements were taken randomly from the middle of the plot rows, and an average height was calculated at the time of harvest.

Table 3.1. Climate data for the 2005 and 2006 growing seasons at Saskatoon Diefenbaker International airport (52° 10.000' N & 106° 43.000' W) and Melfort (52° 49.000' N & 104° 36.000' W), Saskatchewan.

	Location	Year	April	May	June	July	August	September	October
Mean temperature (ºC)	Saskatoon	**2005**	6.4	10.2	14.4	17.5	15.4	11.3	5.2
	Saskatoon	**2006**	8.0	11.7	16.2	20.0	18.0	12.2	1.6
	30 year average		4.4	11.5	16	18.2	17.3	11.2	4.5
	Melfort	**2006**	5.2	10.3	16.0	18.0	16.5	11.0	0.6
	30 year average		2.5	10.8	15.7	17.4	16.4	10.5	3.6
Total Precipitation (mm)	Saskatoon	**2005**	16.0	27.5	160.5	53.5	53.5	74.0	18.0
	Saskatoon	**2006**	38.0	39.8	108.0	32.0	30.0	118.0	32.5
	30 year average		23.9	49.4	61.1	60.1	38.8	30.7	16.7
	Melfort	**2006**	11.0	63.0	73.4	39.2	46.2	119.8	46.9
	30 year average		24.5	45.6	65.8	75.7	56.8	39.9	24.7

Source: Environment Canada (www.climate.weatheroffice.ec.gc.ca)

Near Infrared Spectrophotometer estimation for stem fibre concentration, total oil and protein concentration in the seeds

The straw samples were analyzed for stem fibre content using the Foss 6500 Near Infrared Spectrophotometer (NIR) (Foss NIR Systems Inc. Silver Spring, MD, USA. Model 6500) following the methods outlined by Barton et al. (2002). The NIR instrument determines the physical composition of the sample by measuring log (1/R) values, where R stands for reflectance (Blanco and Villarroya, 2002). The spectra were collected using ISIscan^{TM} software. Straw samples from middle of the stem of about 19 cm length were cut and placed into a sample cup for the NIR scan (Burton, 2007). Visible / near-infrared spectra were obtained in reflectance mode, with a wavelength range of 400-2498 nm. The sample was sub-scanned four times, and the average of the four sub-scans was used for the estimation of fibre concentration. In between each sub-scan, the sample was taken out of the sample cup and shuffled by hand, to ensure that the whole sample would be scanned, and a representative stem fibre concentration was obtained. The essential information from the spectra was extracted by the chemometric technique and multivariate analysis using WinISI software (WinISI^{TM} II calibration development software, Infrasoft Instrument). The average spectra value was compared to a library of 621 known samples to give an estimated stem fibre concentration (%).

Cleaned flax seeds of the RIL population along with the parents and the checks were analyzed for total oil and total protein concentration following calibrations developed at the Crop Development Centre (CDC), University of Saskatchewan, also using the Foss 6500 NIR instrument**.**

Gas-liquid chromatography for estimation of fatty acid profiles

Fatty acid methyl-esters (FAME) concentration was determined by Gas–Liquid Chromatography (GLC). Approximately 100 mg (wet weight) of seed was crushed and transmethylated with 5 mL of 2 % (by volume) H_2SO_4 in methanol at 100°C for 1 h. On cooling, the FAME was collected and mixed thoroughly with 5 mL of hexane. The hexane layer was collected and placed into small GLC vials for measurement. FAME were analyzed by GLC (HP 5890 Series II; Hewlett Packard) using an approach similar to that described by Bhatty and

Rowland (1990). Hewlett-Packard 3385A automation system was used to calculate each fatty acid (palmitic, stearic, oleic, linoleic and linolenic) as percent of the total fatty acids.

Data analysis

Simple statistics of the 2005 Kernen Crop Research Farm trial was performed using untransformed data in the SAS statistical program (SAS Institute Inc. 2005). The analysis of variance for the 2006 multi-location trial was conducted following an examination for normality and homogeneity of error variance using Levene's test. Log-transformed data were used in the analysis for all variables except total oil and plant height characters to assist with convergence and to stabilize the variances over locations (Gomez and Gomez, 1984). To determine main effects and interactions attributable to genotype and location, data were subjected to ANOVA using the PROC MIXED procedure in SAS (SAS Institute Inc. 2005). Least-significant difference (LSD) multiple range test was used to identify specific differences among the 95 RILs when $P < 0.05$ (Mtilsky, 1995). A paired t-test was used to compare significant differences between the parents Viking and E1747; the three checks Hermes, Flanders and Somme; and the potential dual purpose RILs.

The five highest, five lowest and five potential dual purpose RILs were identified using pooled phenotypic data of the 2006 multi-location trial for fibre as well as oil related traits. Correlations and regression analyses were conducted using the 2006 pooled mean untransformed data excluding the parents and the checks (SAS Institute Inc. 2005). Broadsense heritability ($h^2_{b.s}$) of the 2006 multi-location log - tranformed data was also estimated using the GLM procedure in SAS (SAS Institute Inc. 2005).

Results

The flax lines grew well during the crop growing seasons of 2005 and 2006. For the purpose of this study, all agronomic conditions were followed similar to that of the Crop Development Centre Flax Breeding Program, at the University of Saskatchewan, which primarily is aimed to develop good oilseed flax varieties. This procedure was done to ensuure identification of potential high fibre or dual purpose flax lines were more adapted to western Canadian growing conditions as well as more attractive to the conventional oilseed flax growers.

Simple statistics of the 2005 Kernen Crop Research Farm data revealed a wide range of variation among the 95 RILs. Fibre concentration ranged from 15.64 - 23.46 %, plant height from 39.00 - 70.00 cm; total oil concentration from 36.10 – 41.30 %; total protein concentration from 23.20 - 28.10 %; palmitic acid concentration from 5.40– 8.08 %; stearic acid concentration from 2.84 - 5.16 %; oleic acid concentration from 11.21 – 17.42 %, linoleic acid concentration from 19.65 – 76.12 % and linolenic acid concentration from 1.79 – 55.93 % (Table 3.2.1)**.**

A similar range of variation among the 95 RILs was also found in the 2006 multi-location field trial. Fibre concentration in this trial ranged from 15.56 – 21.13 %; plant height from 43.91 – 72.25 cm; total oil concentration from 36.52 – 40.93 %; total protein concentration from 24.02 - 28.23 %; palmitic acid concentration from 5.47 – 7.58 %; stearic acid concentration from 2.86 - 5.11 %; oleic acid concentration from 13.42 – 18.91 %, linoleic acid concentration from 17.55 – 73.38 % and linolenic acid concentration from 1.99 – 56.65 % (Table 3.2.2).

Correlations between the 2005 and 2006 field data were strong for all traits including plant height ($r = 0.81$, $p < 0.0001$), oil concentration ($r = 0.87$, $p < 0.0001$), protein concentration ($r = 0.87$, $p < 0.0001$), linoleic acid and linolenic acid concentration ($r = 0.98$, $p < 0.0001$), indicating similarity in trends between the two years data at the Kernen Research Farm (Table 3.3). The correlation between the 2005 and 2006 fibre concentration data was $r = 0.76$, $p < 0.0001$ at the Kernen Research Farm. Broadsense heritability was estimated for all the traits under study and was high ($h^2_{b.s} \geq 0.80$) for all traits except for fibre concentration, which was found to be moderately ($h^2_{b.s} = 0.63$) heritable (Table 3.4).

Histograms based on the frequency distribution of the 2006 multi-location pooled mean values (Figure 3.1.1, and Appendix Figure A.1) revealed that the fibre concentration, plant

height, total oil concentration, total protein concentration, palmitic acid concentration, stearic acid concentration and oleic acid concentration followed a bell shaped continuous single peak symmetrical distribution. However, linoleic and linolenic acid concentration showed a double peaked frequency distribution (Figure 3.1.2).

Mixed model analysis of variance was performed for the 2006 multi-location field trial. Significant genotypic differences were observed in the pooled data for all traits (Table 3.5) as well as when analyzed for each location separately (Appendix Table A.1). Locations were significantly different from one another for all traits except for palmitic acid concentration and linoleic acid concentration. Genotype by environmental (G × E) interaction was found significant only for protein concentration ($p < 0.0001$), oil concentration ($p < 0.01$) and fibre concentration ($p < 0.01$).

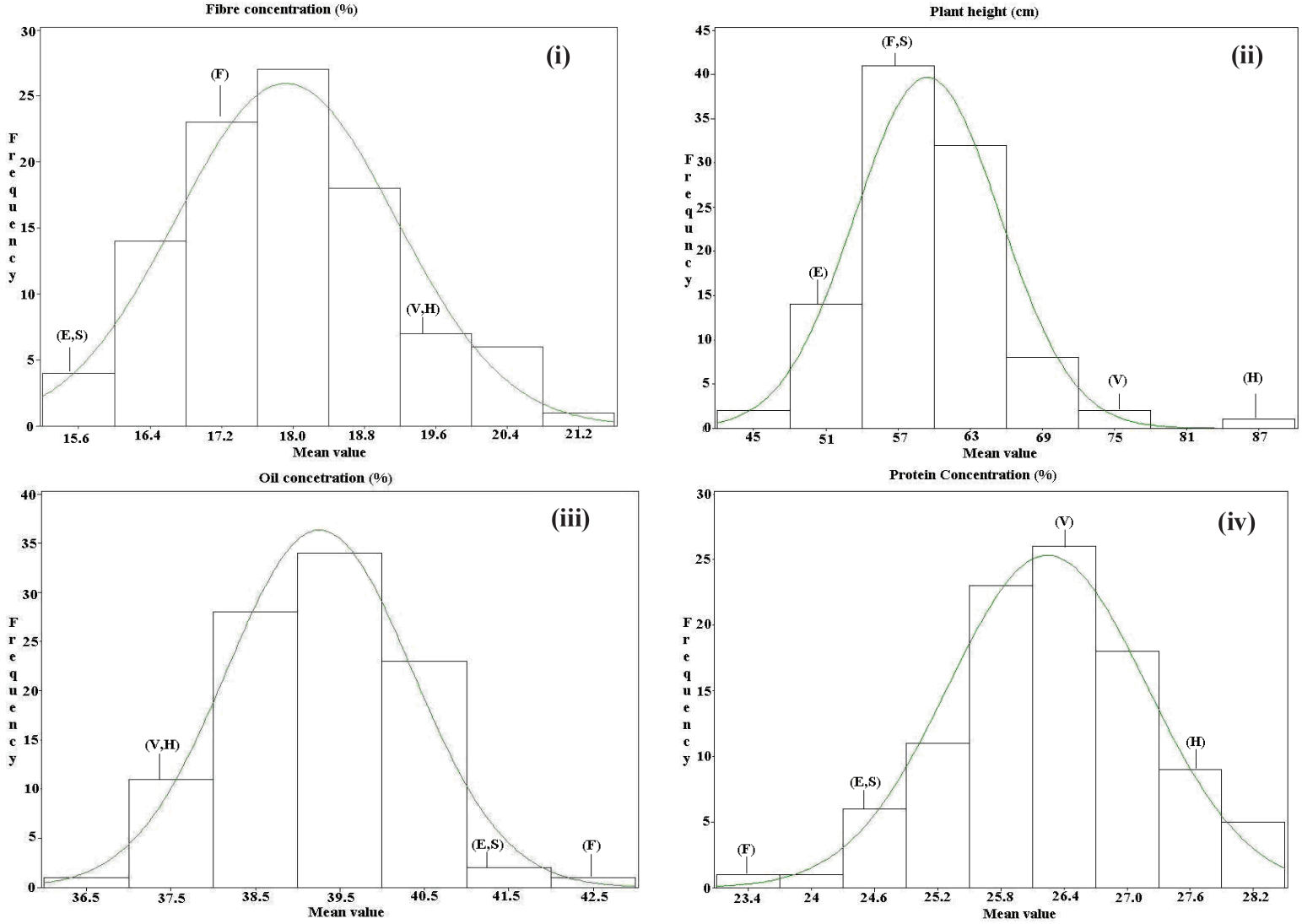


Figure 3.1.1. Histograms and frequency distribution of the RIL population, parents and checks for (i) fibre concentration, (ii) plant height, (iii) oil concentration and (iv) protein concentration grown in the 2006 multi-location field trial. The positions of the parents and the checks on the histograms are represented by their initials, namely Viking (V), E1747 (E), Hermes (H), Flanders (F) and Somme (S).

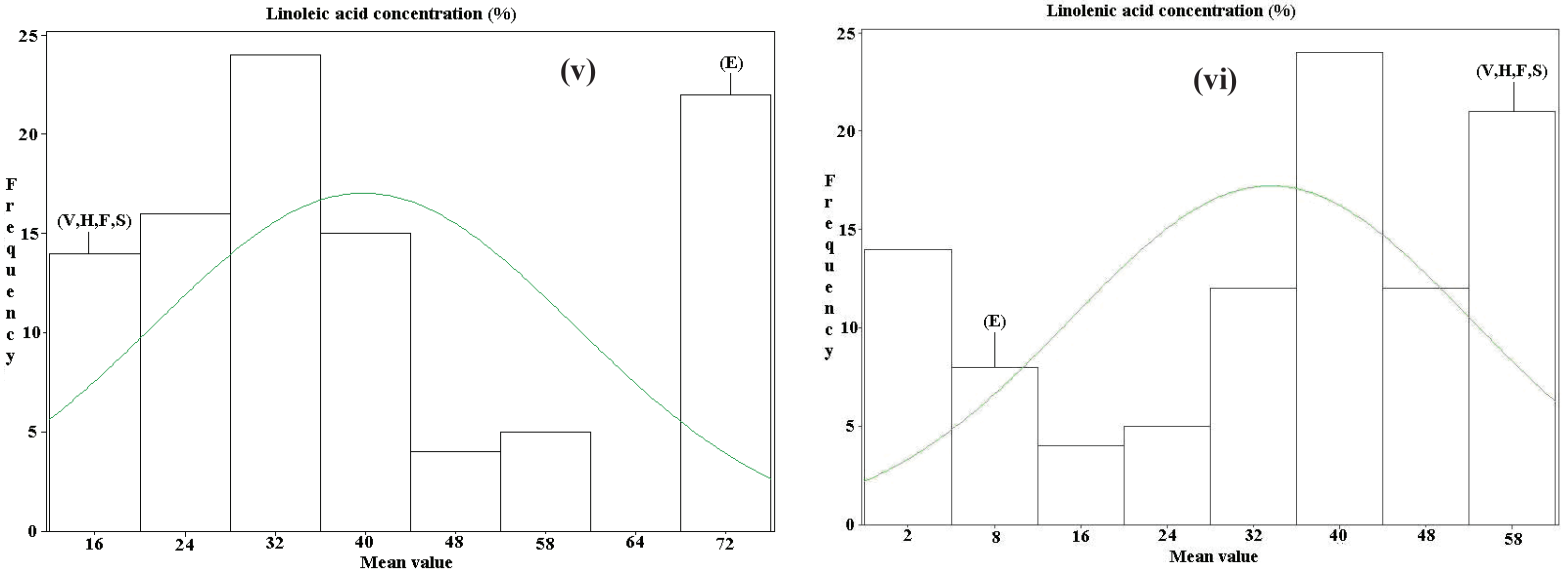


Figure 3.1.2. Histograms and frequency distribution of the RIL population, parents and checks for (v) linoleic acid concentration and (vi) linolenic acid concentration, grown in the 2006 multi-location field trial. The positions of the parents and the checks on the histograms are represented by their initials namely Viking (V), E1747 (E), Hermes (H), Flanders (F) and Somme (S).

Table 3.2.1. Minimum, maximum, standard error of the means (S.E.M), ranges, coefficient of variation (C.V. %) and means of the variables scored across the 95 flax RIL population in 2005 Kernen Research Farm field trial.

Phenotypic traits	Minimum	Maximum	S.E.M.	Range	C.V. %	Mean
Fibre concentration (%)	15.64	23.46	0.14	7.82	7.59	18.36
Plant height (cm)	39.00	70.00	0.62	31.00	11.52	52.45
Oil concentration (%)	36.10	41.30	0.12	5.20	2.94	39.08
Protein concentration (%)	23.20	28.10	0.10	4.90	3.92	25.83
Palmitic acid concentration (%)	5.40	8.08	0.05	2.67	8.32	6.45
Stearic acid concentration (%)	2.84	5.16	0.05	2.33	14.19	3.77
Oleic acid concentration (%)	11.21	17.42	0.10	6.20	6.72	14.70
Linoleic acid concentration (%)	19.65	76.12	1.90	56.46	43.81	42.31
Linolenic acid concentration (%)	1.79	55.93	1.92	54.15	57.21	32.73

Table 3.2.2. Minimum, maximum, standard error of the means (S.E.M.), ranges, coefficient of variation (C.V. %) and means of the variables scored across the 95 flax RIL population in the 2006 multi-location field trial grown at three locations; Melfort, Floral and Kernen.

Phenotypic traits	Minimum	Maximum	S.E.M.	Range	C.V. %	Mean
Fibre concentration (%)	15.56	21.13	0.12	5.57	6.57	17.94
Plant height (cm)	43.91	72.25	0.55	28.33	9.08	59.09
Oil concentration (%)	36.52	40.93	0.10	4.42	2.56	39.20
Protein concentration (%)	24.02	28.23	0.09	4.22	3.38	26.30
Palmitic acid concentration (%)	5.47	7.58	0.04	2.11	6.93	6.31
Stearic acid concentration (%)	2.86	5.11	0.05	2.25	12.45	3.83
Oleic acid concentration (%)	13.42	18.91	0.11	5.49	6.74	16.25
Linoleic acid concentration (%)	17.55	73.38	1.88	55.83	45.32	40.54
Linolenic acid concentration (%)	1.99	56.65	1.87	54.66	55.28	33.01

Table 3.3. Pearson correlation coefficients between the 2005 and 2006 Kernen Research Farm field trials for fibre and oil related variables.

Phenotypic Traits	Correlation coefficient (r)	p-value
Fibre concentration (%)	0.76	$p < 0.0001$
Plant height (cm)	0.81	$p < 0.0001$
Oil concentration (%)	0.87	$p < 0.0001$
Protein concentration (%)	0.87	$p < 0.0001$
Linoleic acid concentration (%)	0.98	$p < 0.0001$
Linolenic acid concentration (%)	0.98	$p < 0.0001$

Table 3.4. Estimates of broad-sense heritability ($h^2_{b.s}$) of different variables for 95 flax RIL population in the 2006 multi-location field trial. Log transformed data were used for all variables except plant height and oil concentration. Mean squares were calculated using GLM procedure using SAS statistical software.

Phenotypic traits	$h^2_{b.s}$
Fibre concentration (%)	0.63
Plant height (cm)	0.84
Oil concentration (%)	0.88
Protein concentration (%)	0.80
Linoleic acid concentration (%)	0.98
Linolenic acid concentration (%)	0.98
Palmitic acid concentration (%)	0.85
Stearic acid concentration (%)	0.86
Oleic acid concentration (%)	0.80

Table 3.5. Variance components along with their F-values for fibre concentration, plant height, oil concentration, protein concentration, linoleic acid concentration and linolenic acid concentration in the 2006 multi-location field trial grown at three locations; Melfort, Floral and Kernen.

Phenotypic traits	df	Fibre concentration (%)	Plant height (cm)	Oil concentration (%)	Protein concentration (%)	Linoleic acid concentration (%)	Linolenic acid concentration (%)
Location	2	208.83**	219.48**	314.89***	95.59**	0.99	8.78**
Genotype	94	9.91***	19.11***	31.36***	30.61**	320.88***	193.18**
Genotype-Melfort	94	3.60***	8.43***	14.92***	21.34***	440.14***	68.22***
Genotype-Floral	94	3.92***	6.70***	9.77***	8.69***	182.05**	88.29***
Genotype-Kernen	94	7.24***	6.68***	11.05***	13.79***	49.37**	51.49***
Genotype × Environment	188	1.65**	1.17	1.45**	1.86***	1.24	1.22

*significance at $p < 0.05$, ** significance at $p < 0.01$, *** significance at $p < 0.0001$.

Performance of parents and checks in the 2006 multi-location field trial

Mean performance of the parents (Viking and E1747) and the three checks is depicted in Table 3.6. Hermes had the highest pooled mean values for fibre concentration (19.87 %), plant height (82.08 cm) and seed protein concentration (27.28 %) among the parents and the checks. E1747 had the highest linoleic acid concentration (69.31 %) among the parents and the checks, whereas Flanders had the highest oil concentration (42.60 %). The highest linolenic acid concentration was found in Somme (57.77 %) and the lowest linolenic acid concentration was found in E1747 (4.38 %).

Among the parents, Viking had significantly higher fibre concentration ($p < 0.01$), plant height ($p < 0.05$), and linolenic acid concentration ($p < 0.01$) as compared to E1747. E1747 had significantly higher oil concentration ($p < 0.05$) and linoleic acid concentration ($p < 0.01$) as compared to Viking. When compared to the checks, Viking had significantly higher fibre concentration as compared to Somme ($p < 0.01$). However, Viking had significantly lower oil concentration as compared to Flanders and Somme ($p < 0.01$). A list of all other comparisons between the parents and the checks can be found in Appendix Table A.2.

Table 3.6. The mean phenotypic values of different fibre and oil related variables for the parents (Viking and E1747) and the checks (Hermes, Flanders and Somme) in the 2006 multi-location field trial grown at three locations; Melfort, Floral and Kernen.

Fibre concentration (%)					**Plant height (cm)**				
Parents & check	**location**			**Mean**	**Parents & check**	**location**			**Mean**
	Melfort	**Floral**	**Kernen**			**Melfort**	**Floral**	**Kernen**	
Viking	18.39	22.01	18.51	19.64	**Viking**	70.00	78.00	68.00	72.00
E1747	15.15	17.08	14.69	15.64**	**E1747**	57.00	60.25	48.75	55.33*
Hermes	18.27	23.02	18.34	19.87	**Hermes**	91.50	90.75	76.00	86.08
Flanders	14.62	21.33	16.13	17.36	**Flanders**	60.75	59.00	53.00	57.58*
Somme	13.46	17.56	14.89	15.30**	**Somme**	66.00	63.50	50.75	60.08
Oil concentration (%)					**Protein concentration (%)**				
Parents & check	**location**			**Mean**	**Parents & check**	**location**			**Mean**
	Melfort	**Floral**	**Kernen**			**Melfort**	**Floral**	**Kernen**	
Viking	38.00	38.45	37.55	38.00	**Viking**	26.34	26.55	25.71	26.20
E1747	41.80	41.25	40.40	41.15*	**E1747**	24.60	24.55	25.40	24.85
Hermes	38.20	38.40	37.40	38.00	**Hermes**	26.75	27.35	27.75	27.28
Flanders	43.15	43.05	41.60	42.60**	**Flanders**	23.20	23.00	24.25	23.48
Somme	41.90	42.40	41.20	41.83**	**Somme**	24.60	23.90	25.00	24.50
Linoleic acid concentration (%)					**Linolenic acid concentration (%)**				
Parents & check	**location**			**Mean**	**Parents & check**	**location**			**Mean**
	Melfort	**Floral**	**Kernen**			**Melfort**	**Floral**	**Kernen**	
Viking	16.45	16.05	17.85	16.78	**Viking**	54.99	55.72	54.41	55.04
E1747	70.53	70.74	66.67	69.31**	**E1747**	4.43	3.96	4.75	4.38**
Hermes	19.11	20.24	19.48	19.61	**Hermes**	52.89	50.45	53.05	52.13
Flanders	15.75	16.85	15.24	15.94	**Flanders**	57.32	54.60	56.83	56.25
Somme	15.63	14.81	14.90	15.11	**Somme**	57.08	58.62	57.60	57.77*

*, **, *** are significantly different from Viking, at 5 %, 1 % and 0.01 % probability respectively; pooled mean values showing no '*' do not differ significantly from Viking according to paired t-test.

Performance of RIL population in the 2006 multi-location field trial

Many transgressive segregants were observed in the RIL population whose values exceeded the parental values. Based on the pooled mean data, the five highest and the five lowest RILs in each category are depicted in Table 3.7.1 and Table 3.7.2.

Fibre concentration:

The fibre concentration in general was found to be higher at Floral as compared to Melfort and Kernen (Table 3.7.1). Five high fibre containing RILs, namely RIL341 (21.13 %), RIL264 (20.78 %), RIL350 (20.53 %), RIL337 (20.31 %) and RIL306 (20.31 %), were selected based on their pooled mean values and had similar ranking positions in each of the three field locations. RIL341 and RIL264 were found to have significantly higher fibre concentration as compared to RIL350, RIL337 and RIL306 ($p < 0.05$).

Similarly, five low fibre containing RILs namely, RIL271 (15.56 %), RIL324 (15.90 %), RIL301 (16.09 %), RIL267 (16.22 %) and RIL260 (16.31 %) were selected based on their pooled mean data. These selected RILs were significantly different from one another but had similar ranking positions at the three locations (Table 3.7.1).

Plant height:

Plant height in general was found to be higher at Floral as compared to Melfort and Kernen (Table 3.7.1). Five tallest RILs selected on the basis of pooled mean values, namely RIL284 (72.25 cm), RIL344 (71.92 cm), RIL283 (68.75 cm), RIL350 (68.42 cm) and RIL293 (67.83 cm) were found to have similar ranking positions at each location. All the five selected RILs were significantly different from one another ($p < 0.05$).

Five shortest RILs, namely RIL297 (43.92 cm), RIL260 (47.17 cm), RIL298 (49.50 cm), RIL256 (49.67 cm) and RIL337 (50.83 cm), were selected on the basis of their pooled mean values. These RILs had similar ranking positions in each location. Except RIL298 and RIL256, all other RILs were found to be significantly different ($p < 0.05$) from one another (Table 3.7.1).

Oil concentration:

Oil concentration of the seeds was found to be comparatively higher at Floral as compared to Melfort and Kernen (Table 3.7.1).The five highest seed oil containing RILs, namely RIL288 (40.93 %), RIL295 (40.80 %), RIL284 (40.80 %), RIL320 (40.72 %) and RIL291 (40.68 %), were selected on the basis of their pooled mean data. The RILs had similar ranking positions at each location. Except RIL295 and RIL284, all other RILs were significantly different from one another ($p < 0.05$). Five lowest seed oil containing RILs, namely RIL350 (36.52 %), RIL280 (37.03 %), RIL342 (37.13 %), RIL338 (37.30 %) and RIL298 (37.37 %), were selected on the basis of their pooled mean. All these five selected RILs were found to have similar ranking positions at each location. Except RIL280 and RIL 342, all other RILs were significantly different from one another.

Protein concentration:

Five high protein containing RILs, namely RIL335 (28.23 %), RIL342 (28.10 %), RIL350 (27.98 %), RIL336 (27.98 %) and RIL298 (27.93 %) were selected on the basis of their pooled mean data from Floral, Melfort and Kernen. These selected RILs were found to be close in their ranking positions at all three locations. Except RIL335 and RIL342 the other three RILs were not significantly different from one another (Table 3.7.1). Similarly, the five lowest protein containing RILs were RIL270 (24.02 %), RIL263 (24.45 %), RIL324 (24.70 %), RIL313 (24.72 %) and RIL345 (24.83 %). These selected RILs were also found to be close in their ranking positions across locations but they were significantly different from one another ($p < 0.05$) (Table 3.7.1).

Linoleic acid concentration:

Among the 95 RILs, high concentrations of linoleic acid were found in RIL327 (73.38 %), RIL271 (72.75 %), RIL273 (71.74 %), RIL333 (71.36 %) and RIL261 (71.11 %) (Table 3.7.2). These RILs had similar ranking positions but did not differ significantly from one another. On the other hand, RIL303 (17.55 %), RIL283 (17.91 %), RIL343 (18.39 %), RIL270 (18.41 %) and RIL350 (18.42 %) were identified having the lowest concentration of linoleic acid. RIL303 and RIL283 had significantly lower linoleic acid concentration as compared to the other three RILs at $p < 0.05$ (Table 3.7.2).

Linolenic acid concentration:

Among the 95 RILs, high concentrations of linolenic acid were found in RIL343 (56.66 %), RIL283 (55.61 %), RIL300 (55.47 %), RIL303 (55.28 %) and RIL255 (54.94 %) (Table 3.7.2). These RILs had similar ranking positions at each of the three locations. Both RIL343 and RIL283 were found to have significantly higher linolenic acid concentration as compared to RIL300, RIL303 and RIL255. RIL308 (2.00 %), RIL309 (2.19 %), RIL327 (2.70 %), RIL273 (2.71 %) and RIL345 (3.00 %) were among the RILs with the lowest concentration of linolenic acid. Except RIL327 and RIL273, the other three RILs were significantly different from one another at $p < 0.05$ (Table 3.7.2).

Other fatty acids

The 95 RILs were also screened for palmitic, stearic and oleic acid concentrations (Appendix Table A.3). The RILs with the highest palmitic, stearic and oleic acid were RIL297, RIL309 and RIL270, respectively. The RILs with the lowest concentration of palmitic, stearic and oleic acid were RIL344, RIL264 and RIL327, respectively. A list of all the five RILs with highest and lowest fatty acid concentration for the above mentioned fatty acids along with their ranking at each field location is depicted in Appendix Table A.3.

Table 3.7.1. The mean phenotypic values of five out of 95 flax RILs with the highest and the lowest values for different fibre and oil related characters in the 2006 multi-location field trial grown at three locations; Melfort, Floral and Kernen.

Fibre concentration (%)									
High RIL	**location**			**Mean**	**Low RIL**	**location**			**Mean**
	Melfort	**Floral**	**Kernen**			**Melfort**	**Floral**	**Kernen**	
341	19.82^{3}	23.23^{7}	20.35^{1}	21.13^{a}	**271**	13.51^{95}	18.11^{93}	15.07^{91}	15.56^{a}
264	21.70^{1}	22.33^{10}	18.31^{15}	20.78^{b}	**324**	13.88^{94}	18.10^{94}	15.72^{79}	15.90^{b}
350	18.26^{6}	24.27^{2}	19.03^{6}	20.53^{c}	**301**	14.57^{90}	18.63^{84}	15.09^{90}	16.09^{c}
337	20.72^{2}	21.75^{15}	18.46^{11}	20.31^{c}	**267**	15.15^{81}	18.73^{83}	14.78^{94}	16.22^{d}
306	18.87^{4}	23.29^{5}	18.79^{8}	20.31^{c}	**260**	15.32^{75}	17.36^{95}	16.26^{77}	16.31^{e}
Plant height (cm)									
High RIL	**location**			**Mean**	**Low RIL**	**location**			**Mean**
	Melfort	**Floral**	**Kernen**			**Melfort**	**Floral**	**Kernen**	
284	74.25^{1}	82.00^{1}	60.50^{1}	72.25^{a}	**297**	45.00^{95}	49.50^{95}	37.25^{95}	43.92^{a}
344	73.25^{2}	82.00^{2}	60.50^{2}	71.92^{b}	**260**	49.25^{94}	52.00^{94}	40.25^{93}	47.17^{b}
283	71.50^{3}	77.00^{6}	57.75^{11}	68.75^{c}	**298**	50.75^{90}	55.00^{93}	42.75^{90}	49.50^{c}
350	69.50^{4}	77.50^{5}	58.25^{10}	68.42^{d}	**256**	49.50^{93}	59.50^{82}	40.00^{94}	49.67^{c}
293	66.25^{11}	78.25^{4}	59.00^{5}	67.83^{e}	**337**	52.00^{88}	57.00^{89}	43.50^{87}	50.83^{d}
Oil concentration (%)									
High RIL	**location**			**Mean**	**Low RIL**	**location**			**Mean**
	Melfort	**Floral**	**Kernen**			**Melfort**	**Floral**	**Kernen**	
288	40.95^{3}	41.75^{2}	40.10^{3}	40.93^{a}	**350**	36.55^{95}	37.15^{95}	35.85^{95}	36.52^{a}
295	40.70^{8}	41.40^{7}	40.30^{1}	40.80^{b}	**280**	37.35^{93}	37.40^{93}	36.35^{92}	37.03^{b}
284	40.90^{4}	41.80^{1}	39.70^{9}	40.80^{b}	**342**	37.60^{90}	37.90^{92}	35.90^{94}	37.13^{b}
320	40.50^{13}	41.40^{8}	40.25^{2}	40.72^{c}	**338**	37.40^{92}	37.95^{91}	36.55^{91}	37.30^{c}
291	40.85^{6}	41.65^{4}	39.55^{15}	40.68^{d}	**298**	37.80^{89}	37.25^{94}	37.05^{88}	37.37^{d}
Protein concentration (%)									
High RIL	**location**			**Mean**	**Low RIL**	**location**			**Mean**
	Melfort	**Floral**	**Kernen**			**Melfort**	**Floral**	**Kernen**	
335	28.50^{1}	27.60^{5}	28.60^{1}	28.23^{a}	**270**	24.45^{95}	23.00^{95}	24.60^{95}	24.02^{a}
342	28.05^{5}	27.70^{3}	28.55^{2}	28.10^{b}	**263**	25.20^{93}	23.00^{94}	25.15^{93}	24.45^{b}
350	27.85^{9}	28.05^{1}	28.05^{7}	27.98^{c}	**324**	25.45^{90}	23.35^{93}	25.30^{92}	24.70^{c}
336	28.20^{4}	27.70^{2}	28.05^{8}	27.98^{c}	**313**	25.60^{86}	23.50^{92}	25.05^{94}	24.72^{d}
298	27.75^{12}	27.70^{4}	28.35^{5}	27.93^{c}	**345**	24.80^{94}	24.35^{80}	25.35^{90}	24.83^{e}

(Values within a column followed by a same letter (s) do not differ significantly according to LSD multiple range test, $p < 0.05$; Values within a column followed by a number is the ranking of the RIL in that particular location)

Table 3.7.2. The mean phenotypic values of five out of 95 flax RILs population with the highest and the lowest values for linoleic acid and linolenic acid concentration in the 2006 multi-location field trial grown at three locations; Melfort, Floral and Kernen.

Linoleic acid concentration (%)

High RIL	location			Mean	Low RIL	location			Mean
	Melfort	Floral	Kernen			Melfort	Floral	Kernen	
327	73.51^{1}	73.11^{2}	73.54^{1}	73.38^{a}	**303**	17.49^{94}	17.44^{95}	17.74^{95}	17.55^{a}
271	73.17^{2}	73.24^{1}	71.84^{2}	72.75^{a}	**283**	17.47^{95}	18.36^{94}	17.92^{92}	17.91^{a}
273	72.84^{3}	72.18^{3}	70.20^{10}	71.74^{a}	**343**	18.49^{91}	18.79^{91}	17.90^{93}	18.39^{b}
333	72.68^{4}	70.43^{10}	70.98^{6}	71.36^{a}	**270**	18.50^{90}	18.84^{90}	17.89^{94}	18.41^{b}
261	71.41^{11}	70.99^{8}	70.95^{7}	71.11^{a}	**350**	17.90^{93}	19.38^{88}	17.97^{91}	18.42^{b}

Linolenic acid concentration (%)

High RIL	Location			Mean	Low RIL	location			Mean
	Melfort	Floral	Kernen			Melfort	Floral	Kernen	
343	57.75^{1}	56.11^{1}	56.13^{1}	56.66^{a}	**308**	1.82^{95}	2.26^{95}	1.92^{95}	2.00^{a}
283	56.62^{3}	54.28^{7}	55.93^{2}	55.61^{a}	**309**	2.24^{93}	2.34^{94}	1.99^{94}	2.19^{b}
300	57.02^{2}	54.06^{10}	55.32^{3}	55.47^{b}	**327**	2.94^{86}	2.79^{89}	2.38^{92}	2.70^{c}
303	56.47^{4}	55.04^{4}	54.34^{4}	55.28^{b}	**273**	2.26^{92}	2.66^{92}	3.23^{84}	2.71^{c}
255	55.57^{7}	55.75^{3}	53.52^{7}	54.94^{b}	**345**	2.59^{88}	2.57^{93}	3.85^{78}	3.00^{d}

(Values within a column followed by a same letter (s) do not differ significantly according to LSD multiple range test, $p < 0.05$; Values within a column followed by a number is the ranking of the RIL in that particular location)

Table 3.8.1. Mean comparisons of the potential dual purpose RILs for different fibre and oil related characters in the 2006 multi-location field trial grown at three locations; Melfort, Floral and Kernen.

Fibre concentration (%)					**t- test**	
Dual RIL	**location**			**Mean**	**Viking (mean)**	**E1747 (mean)**
	Melfort	**Floral**	**Kernen**	**18.92**	**19.64**	**15.64**
319	17.20[22]	22.66[8]	19.05[5]	19.64[a]	ns	*
311	17.97[8]	20.87[36]	19.17[4]	19.34[b]	ns	*
273	18.32[5]	20.77[39]	17.81[27]	18.96[c]	ns	*
309	16.47[44]	20.87[37]	17.87[25]	18.40[d]	ns	ns
287	15.51[70]	22.26[12]	17.08[40]	18.28[e]	ns	ns
Plant height (cm)					**t- test**	
Dual RIL	**location**			**Mean**	**Viking (mean)**	**E1747 (mean)**
	Melfort	**Floral**	**Kernen**	**59.08**	**72.00**	**55.33**
319	56.75[72]	63.50[64]	46.50[76]	55.58[a]	*	ns
311	60.25[50]	65.75[50]	53.75[29]	59.92[b]	*	*
273	57.75[66]	61.50[72]	48.00[71]	55.75[c]	*	ns
309	65.00[18]	69.50[26]	50.75[51]	61.75[d]	ns	ns
287	64.25[21]	68.00[34]	55.00[24]	62.42[e]	ns	***
Oil concentration (%)					**t- test**	
Dual RIL	**location**			**Mean**	**Viking (mean)**	**E1747 (mean)**
	Melfort	**Floral**	**Kernen**	**39.80**	**38.00**	**41.15**
319	40.35[16]	40.40[32]	39.25[24]	40.00[a]	**	ns
311	40.65[10]	41.30[11]	39.50[17]	40.48[b]	*	ns
273	39.30[50]	39.60[60]	38.20[62]	39.03[c]	ns	*
309	39.35[49]	39.45[62]	38.40[54]	39.07[d]	*	**
287	40.65[9]	41.05[17]	39.60[12]	40.43[e]	**	ns
Protein concentration (%)					**t- test**	
Dual RIL	**location**			**Mean**	**Viking (mean)**	**E1747 (mean)**
	Melfort	**Floral**	**Kernen**	**26.42**	**26.20**	**24.85**
319	27.15[24]	26.00[32]	27.10[31]	26.75[a]	ns	*
311	26.65[45]	24.50[77]	26.60[56]	25.92[b]	ns	ns
273	26.25[64]	25.75[39]	26.85[44]	26.28[c]	ns	**
309	27.50[18]	26.70[12]	27.60[17]	27.27[d]	ns	**
287	26.10[69]	25.35[55]	26.25[67]	25.90[b]	ns	*

Values within a column followed by a same letter (s) do not differ significantly according to LSD multiple range test, $p < 0.05$.; Values within a column followed by a number is the ranking of the RIL in that particular location. *, **, *** , are significantly different from Viking and/or E1747, at 5 %, 1 % and 0.01 % probability, respectively; pooled mean values showing 'n.s' do not differ significantly from Viking and/ or E1747 according to paired t-test.

Table 3.8.2. Mean comparisons of the potential dual purpose RILs for linoleic acid and linolenic acid concentration in the 2006 multi-location field trial grown at three locations; Melfort, Floral and Kernen.

Linoleic acid concentration (%)					t- test	
Dual RIL	**location**			**Mean**	**Viking (mean)**	**E1747 (mean)**
	Melfort	**Floral**	**Kernen**	**70.06**	**16.78**	**69.31**
319	70.83[15]	67.31[15]	70.11[12]	69.42[a]	***	ns
311	69.81[19]	68.25[13]	68.47[17]	68.84[a]	**	ns
273	72.84[3]	72.18[3]	70.20[10]	71.74[a]	**	ns
309	71.74[9]	69.89[12]	70.36[9]	70.66[a]	**	ns
287	71.11[14]	66.15[18]	71.69[7]	69.65[a]	***	ns
Linolenic acid concentration (%)					**t- test**	
Dual RIL	**location**			**Mean**	**Viking (mean)**	**E1747 (mean)**
	Melfort	**Floral**	**Kernen**	**3.63**	**55.04**	**4.38**
319	2.46[90]	6.23[79]	2.66[91]	3.78[a]	**	ns
311	4.25[78]	5.40[81]	4.11[76]	4.58[b]	***	ns
273	2.26[92]	2.66[92]	3.23[84]	2.71[c]	***	*
309	2.24[93]	2.34[94]	1.99[94]	2.19[d]	***	***
287	3.88[79]	8.18[76]	2.67[90]	4.91[e]	**	ns

Values within a column followed by a same letter (s) do not differ significantly according to LSD multiple range test, $p < 0.05$.; Values within a column followed by a number is the ranking of the RIL in that particular location. *, **, *** , are significantly different from Viking and/or E1747, at 5 %, 1 % and 0.01 % probability, respectively; pooled mean values showing 'n.s' do not differ significantly from Viking and/ or E1747 according to paired t-test.

Dual purpose RIL selection

Five potential dual purpose lines were selected (RIL319, RIL311, RIL273, RIL309 and RIL287) on the basis of fibre concentration, oil concentration and linolenic acid concentration from the pooled means of the 2006 year multi-location trial (Tables 3.8.1 and 3.8.2).

No significant difference was found between Viking (19.64 %) and the selected dual purpose lines (mean- 18.92 %) for fibre concentration. However, all the dual purpose RILs except RIL309 and RIL287 had significantly higher fibre concentration as compared to E1747 at $p < 0.05$. Plant height of the dual purpose RILs was found to be more similar to E1747 (55.33 cm) as compared to Viking (72.00 cm). The selected dual purpose RILs were significantly different from one another for both fibre concentration and plant height.

Oil concentration of the dual purpose RILs (mean- 39.80 %) was found to be intermediate between Viking (38.00 %) and E1747 (41.15 %). Four of the five RILs had higher oil percentage as compared to Viking ($p < 0.05$). Three out of five RILs (RIL319, RIL311, RIL287) had similar oil concentration when compared to E1747. Protein concentration of the potential dual purpose RILs was significantly higher than E1747 (24.85 %), except for RIL311. However, they were all similar to the Viking parent (26.20 %) in protein concentration (Table 3.8.1).

Linoleic acid concentration of the potential dual purpose RILs was significantly higher ($p < 0.01$) than Viking (16.78 %) but were similar to E1747 (69.31 %). None of these selected RILs had any significant difference among themselves with respect to linoleic acid concentration (Table 3.8.2). Out of the five RILs, three (RIL319, RIL311 and RIL287) had no significant difference from E1747 (4.38 %) for linolenic acid concentration. In addition, RIL309 had significantly less ($p < 0.0001$) concentration of linolenic acid as compared to E1747. All these RILs had significantly lower linolenic acid concentration as compared to Viking ($p < 0.01$). The above mentioned RILs were all significantly different from one another for linolenic acid concentration. The performance of the potential dual purpose RILs for other fatty acids such as palmitic, stearic and oleic acid concentration is depicted in Appendix Table A.4.

Correlation and Regression

Correlation analysis

The relationships among fibre concentration, plant height, oil concentration, protein concentration and fatty acid concentrations were investigated using correlation analysis of both single location data at the Kernen Crop Research Farm in 2005 as well as pooled mean data of the 2006 multi-location field trial. Pearson correlation coefficients of all the traits along with their significance values are depicted in Table 3.9. Parents and checks were excluded from the correlation calculation as they could influence the correlation values due to the diverse nature of their stem fibre and seed oil characteristics.

In the 2006 multi-location field trial, a positive correlation was found between fibre concentration and plant height ($r = 0.43$, $p < 0.0001$). Positive correlations were also found between fibre concentration and oil concentration ($r = 0.25$, $p < 0.01$) as well as between fibre concentration and linolenic acid concentration ($r = 0.13$, $p < 0.01$). A negative relationship was found between fibre concentration and total protein concentration ($r = - 0.33$, $p < 0.01$). Similarly, a negative correlation was also found between fibre concentration and linoleic acid concentration ($r = -0.13$, $p < 0.01$). However, no significant correlations were found between fibre concentration and any of the traits in the 2005 year Kernen Research Farm field trial (Table 3.9).

Plant height apart from being correlated with fibre concentration was also found positively correlated with oil concentration ($r = 0.28$, $p < 0.0001$) and linolenic acid concentration ($r = 0.24$, $p < 0.0001$). Plant height was found negatively correlated with total protein concentration ($r = -0.24$, $p < 0.0001$) and linoleic acid concentration ($r = -0.23$, $p < 0.0001$) (Table 3.9). Similar results were also obtained in the 2005 Kernen Research Farm field trial, however the magnitude of significance was lower than the 2006 multi-location field trial. A list of correlations between other fatty acids is depicted in Table 3.9 and Appendix Table A.5. In addition, a list of all correlations for fibre and oil related traits analyzed separately for each field location in the 2006 multi-location trial is depicted in Appendix Table A.6.

Table 3.9. Pearson correlation coefficients of 2005 and 2006 field trials for fibre and oil related variables.

Phenotypic Traits		Fibre concentration (%)	Plant height (cm)	Oil concentration (%)	Protein concentration (%)	Linoleic acid concentration (%)
Plant height	$r_{(M,F,K)}$	0.43***				
	r_k	0.12				
Oil concentration (%)	$r_{(M,F,K)}$	0.25**	0.28***			
	r_k	0.05	0.30**			
Protein concentration (%)	$r_{(M,F,K)}$	-0.33**	-0.24***	-0.76***		
	r_k	-0.01	-0.26*	-0.79***		
Linoleic acid concentration (%)	$r_{(M,F,K)}$	-0.13**	-0.23***	-0.02	0.07	
	r_k	-0.17	-0.20*	-0.11	0.15	
Linolenic acid concentration (%)	$r_{(M,F,K)}$	0.13**	0.24***	0.03	-0.08	-0.99***
	r_k	0.16	0.21*	0.11	-0.14	-0.99***

*significance at p= 0.05, ** significance at p= 0.01, *** significance at p= 0.0001, n.s = not significant. '$r_{(M,F,K)}$' denotes Pearson correlation coefficient of pooled mean data of 2006 multi-location field trial; 'r_k' denotes Pearson correlation coefficients of mean data of 2005 Kernen Research Farm field trial. All correlations have been calculated using untransformed data excluding the parents and the checks.

Regression analysis

Linear regression analysis was conducted where significant correlations were found with regard to fibre concentration, such as with plant height, oil concentration, protein concentration, linoleic acid concentration and linolenic acid concentration.

The mean values of traits like plant height, oil concentration, protein concentration, linolenic acid and linoleic acid concentration were plotted against fibre concentration values and are shown clustered about the regression line in Figures 3.2.1 and 3.2.2. The regression line was drawn from the regression equation, in which X= fibre concentration (independent varable) and, Y= other traits (dependant variable). Regression analysis for both 2005 and 2006 year field trials revealed non significant and essentially a zero regression coefficient of determination R^2 (%) values between fibre concentration and traits such as plant height, oil concentration, and protein concentration. However, regression analysis was found significant, though very low between fibre concentration and linoleic acid concentration ($R^2 = 6$ %, $p < 0.05$); and between fibre concentration and linolenic acid concentration ($R^2 = 6$ %, $p < 0.05$).

As expected, the regression coefficients between linolenic acid concentration and linoleic acid concentration ($R^2 = 99$ %, $p < 0.0001$); and between oil concentration and protein concentration ($R^2 = 50$ %, $p < 0.0001$) were found to be significant (Figure 3.2.2).

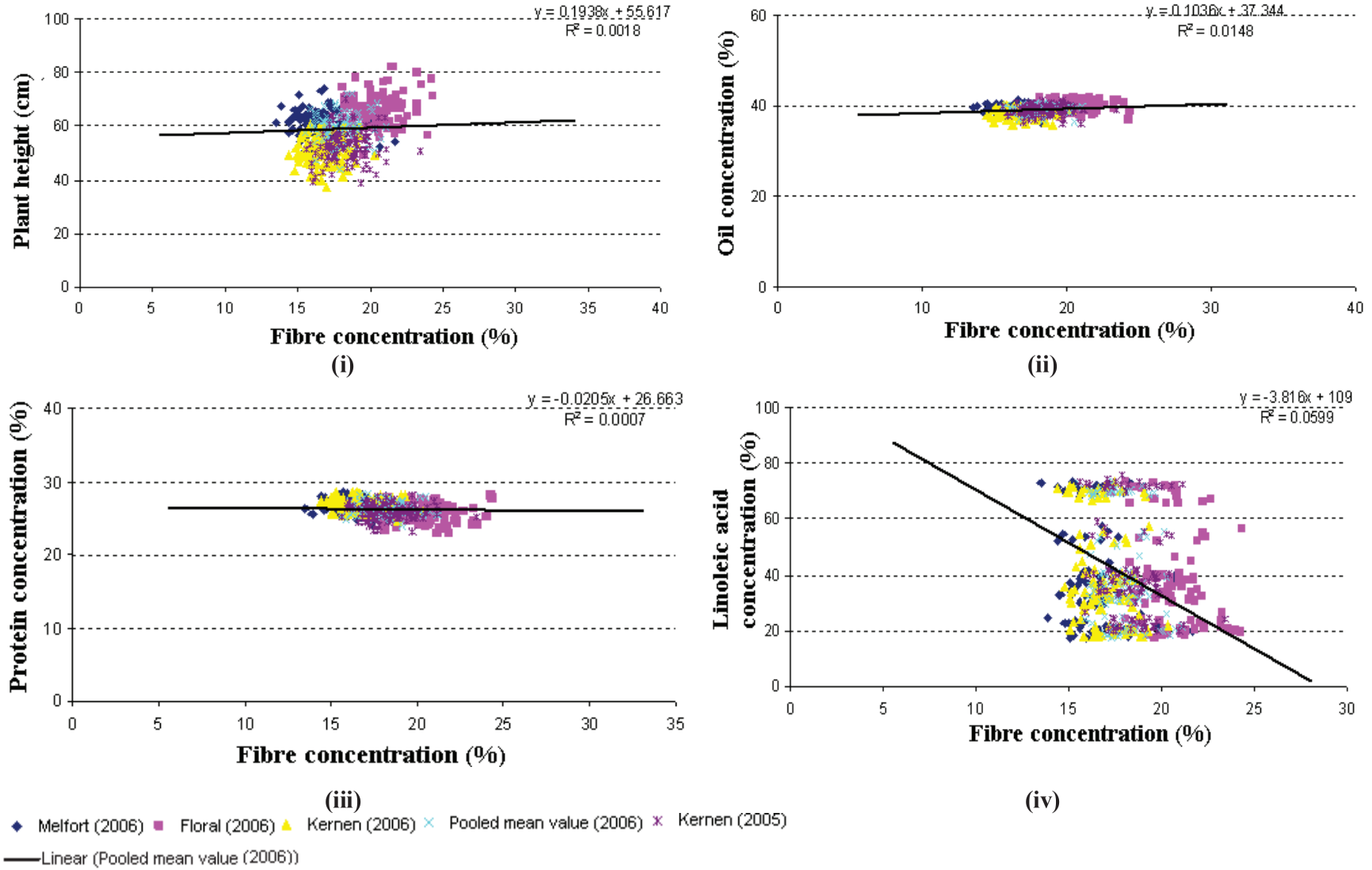


Figure 3.2.1. Regression graph of (i) plant height on fibre concentration, (ii) oil concentration on fibre concentration, (iii) protein concentration on fibre concentration and (iv) linoleic acid concentration on fibre concentration. Regression coefficient (R^2) and regression equation (y) were calculated using 2006 multi location pooled field data.

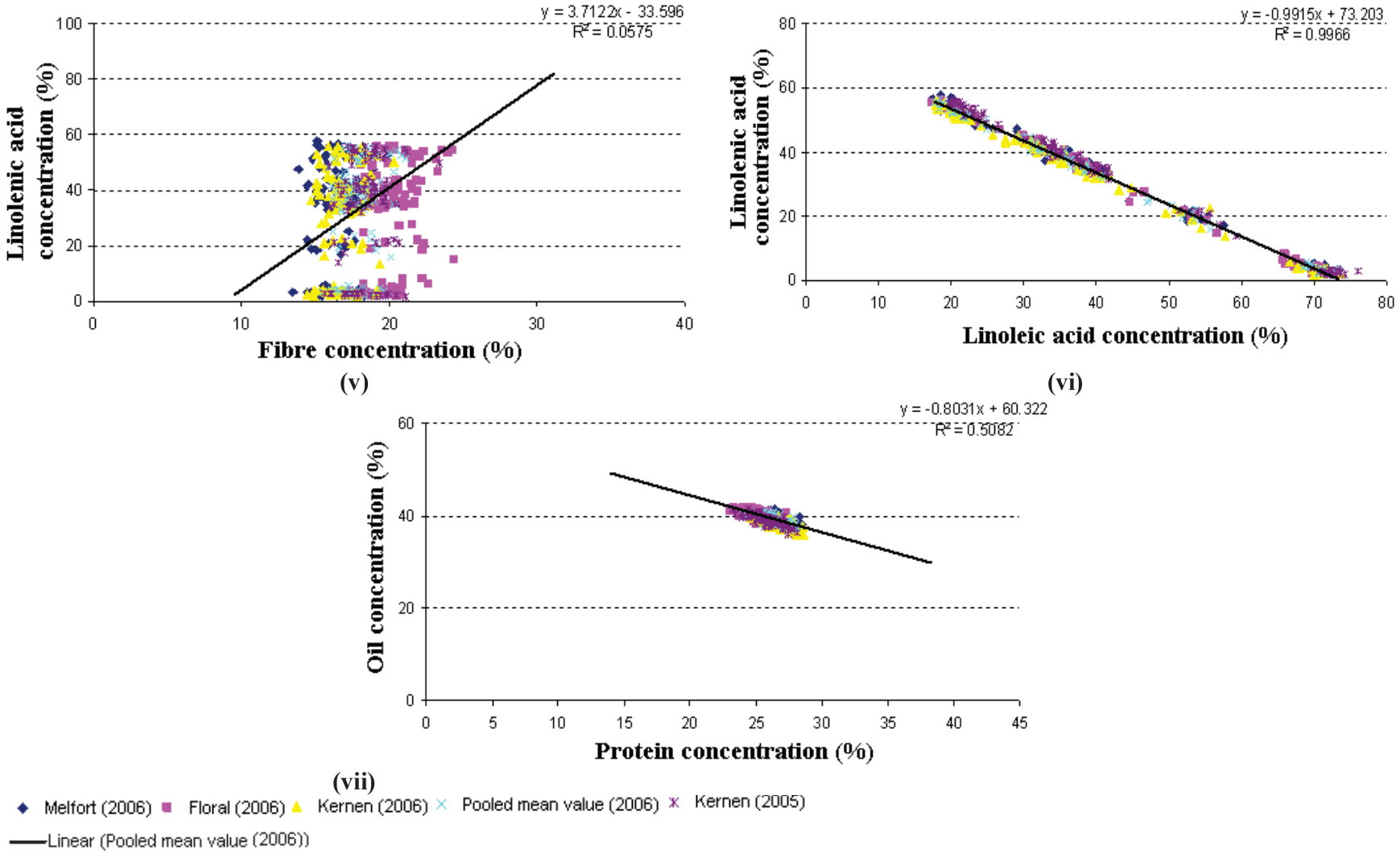


Figure 3.2.2. Regression graph of (v) linolenic acid concentration on fibre concentration, (vi) linolenic acid concentration on linoleic acid concentration, and (vii) oil concentration on protein concentration. Regression coefficient (R^2) and regression equation (y) were calculated using 2006 multi location pooled field data.

Discussion

A primary constraint to the establishment of fibre based industries in western Canada is the lack of knowledge about fibre production and its genetic architecture. This study investigated, for the first time in western Canada, the field performance of a 95 member RIL population derived from a cross between European fibre flax cultivar Viking and an oilseed flax genotype E1747.

Frequency distribution of the population revealed that fibre concentration, plant height, total oil concentration and protein concentration were quantitatively inherited and were under the influence of several minor genes (Figures 3.1.1 and 3.1.2). These observations are in line with a previous study by Keijzer and Metz (1992), who indicated fibre concentration as a multigenic trait. Similarly, studies in flax seed oil concentration (Kenaschuk, 1975a, b; Comstock, 1966; Comstock et al., 1969; Rowland and Bhatty, 1987) as well as in other crops such as mustard and canola (Gunasekera et al., 2006) have found a quantitative nature of inheritance for oil and protein concentration. Conversely, fatty acids such as linoleic acid and linolenic acid concentration showed a double-peaked frequency distribution confirming the earlier reports that these traits were qualitatively inherited and were controlled by two major genes (Rowland and Bhatty, 1990).

The wide range of variation found in fibre concentration, plant height and oil related traits in the parents, checks and the 95 RILs (Tables 3.2.1 and 3.2.2) suggested that breeders could choose genotypes on the basis of stem fibre as well as oil related traits and thereby respond effectively to different market needs. As variability in a population is the key for any trait improvement, Viking × E1747 population (fibre × oilseed) is highly suitable for selecting lines for stem fibre, seed oil or dual purpose traits. A similar study by Foster et al. (1997) also suggested that crossing oilseed × oilseed and oilseed × fibre varieties would constitute better source materials for breeding high yielding dual purpose cultivars as compared to crossing fibre × fibre type cultivars.

Kaul et al. (1994) reported that the bast fibre concentration of their oilseed cultivars varied on an average from 7.1 % to 9.8 % in Germany, whereas Sankari (2000a) in Finland found that the bast fibre content of the oilseed flax stem was 16.9 %. In the present study, the

average fibre concentration of the oilseed parent and the oilseed checks were found to be similar (average -16.1 %) to that of Sankari (2000a).

Many transgressive segregants have also been found in Viking × E1747 RIL population, which could be utilized for future flax breeding purposes including dual purpose flax variety development (Tables 3.7.1 and 3.7.2). Interstingly, fibre concentration of all five high fibre RILs (RIL341, RIL264, RIL350, RIL337 and RIL306) were found to exceed the fibre parent Viking. All the five high oil containing RILs (RIL288, RIL295, RIL284, RIL320, RIL291) had oil concentration close to the oilseed type parent (E1747). Many of the selected RILs (RIL308, RIL309, RIL327, RIL273, RIL345) had similar linolenic acid concentration as compared to E1747, indicating the possibility of selecting lines for specific end use purposes.

Genotype × Environment interaction (G × E)

Multi-location field trial analysis of variance at three locations namely, Melfort, Floral and Kernen, revealed significant differences between the genotypes for all the traits (Table 3.5). A significant G × E interaction ($p < 0.01$) was observed for fibre concentration in the present study (Table 3.5). Similar studies on fibre yield conducted by Easson and Long (1992b) and Keijzer and Metz (1992) showed significant effect of environment on this trait. Easson (1989) reported fibre content to be affected (decreased) when sowing was delayed until after mid –May. According to Scheer-Treibel et al. (2000), bast fibre morphology and mechanical properties largely was dependant on the growing conditions leading to a large environmental component of variance in fibre characteristics. However, no G × E interaction between genotypes and sites was observed in a study by Foster et al. (1997) for fibre yield based on 18 oilseed flax and 10 fibre flax varieties grown at the University of Birmingham, UK.

It is also worth mentioning that even though there was a significant G × E interaction for fibre concentration, the fibre flax parent Viking and fibre flax check Hermes had consistently the highest fibre content over all three locations in the 2006 multi-location field trial (Table 3.6). Furthermore, there were no drastic changes in the ranking positions of the RILs for fibre concentration across locations (Table 3.7.1), indicating the absence of cross over interaction (Gregorius and Namkoong, 1986).

Similarly, plant height was also found to be consistently high in the cultivar Hermes followed by Viking in all the locations. Among the Kernen, Melfort and Floral locations, plant height was found tallest in the Floral location. Though there were significant differences between genotypes as well as between locations, there was no significant G × E interaction for plant height in the current study (Table 3.5). This outcome was supported by Dimmock et al. (2005) where the authors found non-significant variety by year interaction for plant height in fibre flax.

Oil concentration and protein concentration are important characteristics for breeding oilseed flax varieties in western Canada. Previously, studies on oil and protein concentrations in flax have shown quantitative inheritance and their being affected by environmental factors such as soil, presence of diseases, as well as agronomic practices of nitrogen application, irrigation, date of sowing and temperature (Comstock, 1966; Comstock et al., 1969; Green and Marshall, 1981; Green 1986; Kenaschuk, 1975a, Oomah and Kenascuk, 1995; Couture et al., 2002). Significant G × E interaction was also found for oil ($p < 0.01$) as well as for protein concentration ($p < 0.0001$) in this study, confirming the influence of environment on the above mentioned traits (Table 3.5).

For all other traits such as palmitic, stearic, oleic, linoleic and linolenic acid concentration, no G × E interaction was found significant, indicating the stability of these traits across locations and environments. Green (1986), however, found that high temperatures affected the relative proportions of unsaturated fatty acids in most genotypes.

Heritability of all traits under this study was found to be high ($h^2_{b.s} \geq 0.80$) except for fibre concentration, which was found to be moderately heritable ($h^2_{b.s} = 0.63$) (Table 3.4). This result indicated that selection for fibre content will be comparatively difficult. On the contrary, a high heritability ($h^2 = 0.92$) was observed for fibre content in a similar RIL population derived from Viking × E1747 in the study conducted in the Netherlands by Vromans in 2006. In addition studies in Europe by Fouilloux (1989) and Popescu et al. (1995, 1998) also found high heritability for flax fibre concentration. Due to these inconsistencies, further research may be needed to investigate the effect of G × E on fibre concentration in order to improve the efficiency of selection for breeding high fibre oilseed lines.

Correlations and regression analysis

This study showed a positive correlation ($r = 0.43$, $p < 0.0001$) between fibre concentration and plant height (Table 3.9). A similar study by Burton (2007) suggested a positive and significant correlation between fibre concentration and plant height. Studies by Kaul et al. (1994) and Horne et al. (2008) also reported a positive correlation between plant height and fibre yield of flax. The positive correlation between plant height, fibre concentration, oil concentration, and linolenic acid concentration, shows the importance of adequate vegetative growth needed for good fibre, as well as for better oil production. A significant negative correlation was found between fibre concentration and protein concentration and between fibre concentration and linoleic acid concentration. However, all the correlations between fibre concentration and other traits were negligible when each location was analyzed separately (Table 3.9, Appendices Table A.5 and Table A.6). Another study by Kaul et al. (1994) also indicated a negligible negative correlation between fibre content and oil yield. This correlation is crucial as seed oil will always be the main economic product harvested from oilseed flax, and straw fibre will be a secondary product.

A regression study for fibre concentration with plant height, oil concentration and protein concentration showed very low regression coefficient value, indicating the lack of cause and effect relationship and failure to predict the variabilities based on the fibre concentration (Figure 3.2.1 and 3.2.2). Regression coefficient R^2 (%) values for fibre with respect to linolenic and linoleic acid concentration were significant ($p < 0.05$). However, regression coefficient ($R^2 = 6$ %) being very low is insufficient to predict the variability in both these fatty acid concentration based on the fibre content measurements. This lack of both strong correlation and regression between fibre concentration and other oil related traits may be because these lines were not selected for stem fibre. The above mentioned results support the findings of earlier researchers (Foster et al., 1997, 1998, 2000; Sankari, 2000a; Dribnenki, 2008) that fibre concentration can be improved in the short Canadian oilseed varieties without affecting seed oil characteristics.

Dual purpose flax selection

Dual purpose flax varieties are productive for both stem fibre and seed oil extraction and can increase diversification and viability of this crop in North America. However, attempts reported to date in order to find viable dual-purpose varieties have met with only limited success (Foster et al., 2000; Dimmock et al., 2005). The previous oilseed and dual-purpose varieties have not been able to match fibre flax varieties for fibre yield (Dimmock et al., 2005) and there may be physiological reasons for expecting some degree of compensation between seed and fibre components (Burton, 2007).

Easson and Molloy (2000) previously pointed out that oilseed flax can yield only about 1/3 the fibre yield of fibre flax, whereas fibre flax can yield 60 % of the seed yield of oilseed varieties, so selection for dual purpose varieties should be based on fibre flax varieties. However, others have recognized that the seed component has been the most important economically, and considering that fibre length and fineness is less important for many modern applications such as biocomposites, pulp and paper, opted for oilseed, or oilseed × fibre flax as parents for breeding dual purpose varieties (Foster et al., 1997, 1998, 2000; Sankari, 2000a). In addition, Foster et al. (1997) found less variation for the important traits in fibre flax cultivars as compared to oilseed flax, indicating better chances of improvement in oilseed type cultivars. He considered a hybrid approach for dual-purpose varieties but found insufficient heterosis for producing hybrid seed on a commercial scale. Therefore, he suggested development of recombinant inbred lines (RIL) produced from crossing oilseed or oilseed × fibre flax as parents for breeding dual purpose varieties.

The Crop Development Centre (CDC) at the University of Saskatchewan is attempting to increase the straw fibre concentration in the Canadian oilseed flax cultivars by using a fibre x oilseed (Viking × E1747) derived RIL population. The present investigation has identified five potential RILs based on pooled data over multiple locations, namely RIL319, RIL311, RIL273, RIL309 and RIL287 (Tables 3.8.1 and 3.8.2). All these RILs had fibre concentration comparable to the fibre flax parent Viking, and had good edible oil characteristics similar to E1747. Other oil characters such as protein concentration and fatty acid profile of these selected RILs were intermediate between the two parents and hence could be of potential value for a future dual purpose flax breeding program.

In conclusion, this study is the first step into screening for dual purpose traits in western Canada using a RIL population derived from a cross between fibre × oilseed type flax. A wide range of variability present in the population has allowed selection of high fibre lines as well as lines with unique seed oil and fatty acid profiles (Tables 3.7.1, 3.7.2 and Appendix Table A.4). Based on correlation and regression studies (Table 3.9), it should be possible to breed for high stem fibre concentration in flax without impacting on any seed oil related traits. However, the study also shows the influence of environment in controlling fibre concentration as well as oil and protein concentration confirming earlier reports that several minor genes are controlling these characters (Table 3.5). Therefore, further studies are needed to evaluate the suitability of the potential high fibre and dual purpose lines over several locations and years in order to develop stable varieties or for use as parents in the future crossing programs.

CHAPTER 4

Anatomical characterization of flax (*Linum usitatissimum* L.) stem with special reference to bast fibre production

Abstract

Eleven diverse genotypes from a subset of a 95 entry recombinant inbred line (RIL) population derived from a cross between a fibre flax (Viking) and an oilseed flax genotype (E1747- an EMS mutant) along with the parents and a check (Hermes) were used in the flax anatomy study. Differences in the stem anatomy of diverse genotypes were identified using a completely randomized design with three replications each in the field as well as under the controlled environment. Stem cross-sections using an optical microscope were analyzed at three different growth stages, namely seedling, green capsule and physiological maturity; and for ten different anatomical regions. The results from the study supported the use of controlled environments, such as growth chambers, for the purpose of quick screening of high fibre containing genotypes, especially at the green capsule stage of plant growth. The results also indicated that high fibre flax lines could be selected based on anatomical markers such as the average area of single fibre cells, number of fibre cells, total fibre area and fibre to stem area ratio in diverse flax lines.

Introduction

Flax is an annual crop that is a source for both oil and fibre in the form of seed oil and stem fibre. Recent trends towards environmentally friendly alternatives to petrochemical-derived products has led to increased interest in the value of flax fibres as a renewable plant resource, especially for bio-composites, pulp and paper (McDougall et al., 1993). It is known that product quality and productivity can be improved by a better understanding of material structure (Roland and Vian, 1991). In order to improve the fibre production in flax varieties, it is very important to know the ultrastructure of the fibre bearing stem tissues in detail.

In plant anatomy, a fibre is defined as a long, tapering schlerenchyma cell, which does not transport water (Mauseth, 1988). Flax fibres originate from the procambial cells in the protophloem; they are long and multinucleate cells without septum or partition (Esau, 1943, 1977). These fibres have a length of 2-5 cm with 5-15 μm thickness of secondary cell walls and

are gathered in bundles that encircle the vascular cylinder. Matured fibre bundles consist of 12-40 individual cells on average, depending upon the flax variety used (Esau, 1943). During plant growth, fibres develop in two main steps (a) cell elongation and (b) thickening of secondary cell walls (Gorshkova et al., 1996). The lifecycle of flax plants consists of 12 distinct growth phases of which 45-60 days constitutes the vegetative phase, 15 - 25 days the flowering phase and 30-40 days the seed maturation phase (Flax Council of Canada, 2008). Fibre differentiation, characterized by deposition of a cellulose-rich secondary wall, starts very early during flax development (Andeme-Onzighi et al., 2000) and continues throughout all plant growth stages.

Flax varieties grown for seed oil (oilseed) have short stems with multiple branches and seed capsules. In contrast, fibre flax varieties have long, slender and unbranched stems (Hayward, 1948). The overall bast fibre content of the oilseed variety stems has been found to be 11-15 % (Burton, 2007) whereas the overall bast fibre content of the fibre flax varieties has ranged from 17-35 % (Booth et al., 2004). Very limited studies have been done to characterize the differences between fibre flax and oilseed flax with respect to stem anatomy (Dijon, 2002; His et al., 2001) and fibre ultrastructure (Fröier, 1960; Sankari, 2000a) as compared to other fibre crops such as cotton, ramie and jute. Moreover, relationships between anatomical characteristics of flax stems and fibre yield / content are not known. According to Scheer-Triebel et al. (2000), bast fibre morphology and mechanical properties largely depend on growing conditions. Thus, it is also important to understand which particular anatomical traits may be influenced by environment and affect fibre deposition.

Quantification of fibre characteristics is very laborious and time consuming. Laboratory spectroscopic methods such as near infrared spectroscopy (NIR) are currently being used to estimate the fibre content of plants (Barton et al., 2002). This procedure requires growing hundreds of plants in the field, harvesting them and cutting the stems to fit a sample cup (19 cm in length) for determining spectral measurements. It will be interesting to know if relationships between anatomical characters and fibre content as measured by NIR exist, which may help in selecting potential high fibre lines quickly and efficiently. Therefore, the objective of this study was to first understand the anatomical basis of variation in fibre content between diverse flax types at different growth stages and environments such as the field and growth chamber. The

second objective was to identify anatomical markers for quick and easy selection of high fibre genotypes.

Material and methods

Genotype selection

Flax genotypes with varying fibre content were estimated by near infrared spectroscopy (NIRS) based on multi-location field trials in 2006 at three Saskatchewan locations, namely Melfort, Kernen and Floral (Chapter 3). A subset of the recombinant inbred lines (RILs) derived from Viking (fibre flax) × E1747 (oilseed flax) along with the parents and a check (Hermes: a fibre flax cultivar) were used for this study. The genotypes were selected on the basis of mean fibre concentration, plant height, oil concentration and linolenic acid concentration values from the 2006 multi-location field trial data (see Chapter 3). A detailed description of all the selected RILs and cultivars / lines are shown in Table 4.1.

Experimental design

Plants were sown in a completely randomized design with three replications at the Kernen Crop Research Farm near Saskatoon during May, 2007 as well as in growth chambers at the University of Saskatchewan (Figures 4.1.1 and 4.1.2). In the growth chamber, the plants were grown at 25^{o}C day and 17^{o}C night with 18 h light / 6 h dark cycle. Growth chamber plants were fertilized twice, once during sowing and once at the onset of flowering with 15-30-15 all purpose N-P-K pot fertilizer (Plant Prod, Brampton, ON, Canada) according to the manufacturer's instruction, in Sunshine Mix G3 soil. The plants grown at the Kernen Farm did not receive any fertilizers or pesticides or supplemental irrigation. The plants were sown using a small plot seeder with 30 cm row spacing. The plots had a 3.6 m row length with a planting density of 400 seeds m^{-2}.

Stem segments of about 5 cm length were collected in three replicates from the field and from three different pots in the growth chamber at three growth stages, namely seedling (20 days after germination), green capsule and physiological maturity.

Table 4.1. List of selected flax cultivars and recombinant inbred lines (RILs) derived from Viking × E1747 used for the flax anatomical study.

Sl No.	Accession	NIR fibre content (%)	Plant height (cm)	Oil %	Linolenic acid %	Classification
1	**Viking**	19.64	72.00	38.00	55.04	Fibre flax parent
2	**Hermes**	19.87	86.08	38.00	52.13	Fibre flax check
3	**E1747**	15.64	55.33	41.15	4.38	Oilseed flax parent
4	**H341**	21.13	59.83	39.87	51.26	
5	**H350**	20.52	68.42	36.52	54.61	High fibre % RILs
6	**H337**	20.31	50.83	39.75	34.69	
7	**L271**	15.56	57.50	39.80	3.05	
8	**L260**	16.31	47.17	39.57	4.18	Low fibre % RILs
9	**L267**	16.22	53.92	38.25	37.09	
10	**D311**	19.34	59.92	40.48	4.58	Potential dual purpose RILs (having good stem fibre % as well as good edible oil characteristics)
11	**D273**	18.96	55.75	39.03	2.71	
12	**D319**	19.64	55.58	40.00	3.78	
13	**S268**	19.92	51.91	40.10	52.31	Short stature RIL
14	**T284**	18.38	72.25	40.80	40.01	Tall stature RIL

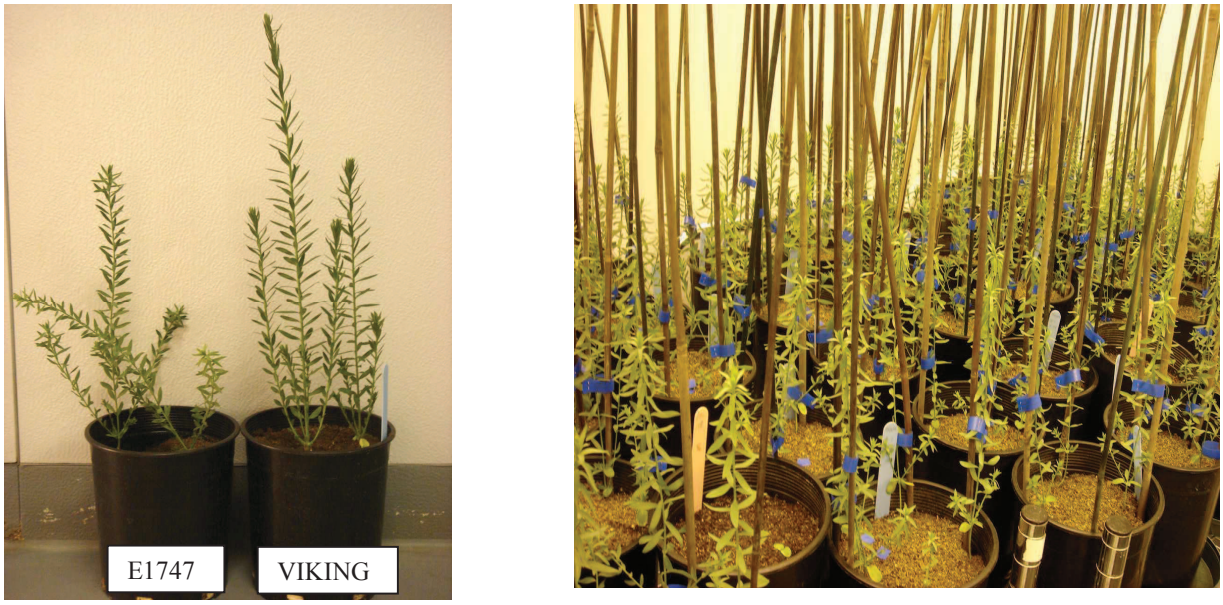


Figure 4.1.1. E1747 and Viking plants at seedling stage growing in the growth chamber study in 2007.

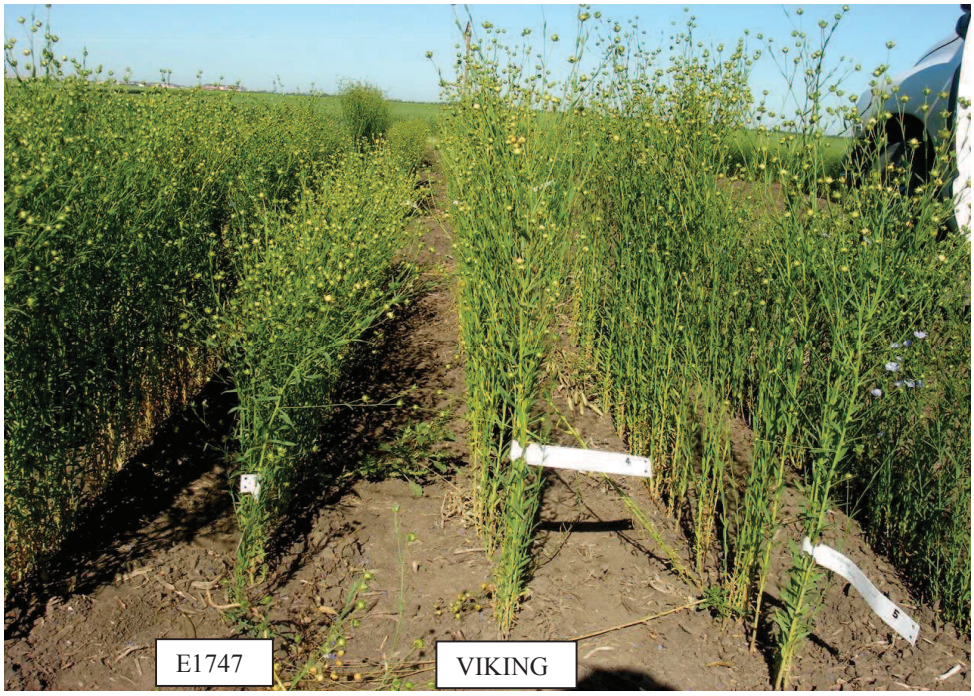


Figure 4.1.2. E1747 and Viking plants at the physiological maturity stage growing at the Kernen Crop Research Farm in 2007.

Comparative analysis of the stem sections

Hand cut stem cross-sections were prepared using platinum chrome double edge blades of approximately 0.1 mm thickness from the second or third basal internode at the seedling stage and from internode eight- nine for the other growth stages. The stem sections were preserved in formalin-aceticacid- alcohol solution (Johansen, 1940) for future laboratory analysis. The stem sections were stained with Toluidine Blue O (0.1 %) for 30 sec and excess stain removed by repeated washing with distilled water. Sections were immediately observed using a Carl Zeiss Axioskop 40 optical microscope with 10 x magnification. All stem sections were projected to this constant magnification. Images were taken with a pixel link color microscopy camera (model PL-A686C) attached to the microscope. The area of various tissue types were analyzed digitally by tracing, using Carl Zeiss imaging system (Axiovision 29A software 4.6.3, 2007).

Ten anatomical parameters were measured in the diverse flax lines, namely epidermis width (µm), cortex width (µm), cambial zone width (µm), average area of single fibre cell (μm^2), number of fibre cells in the stem, total fibre area in the stem (μm^2), pith area (μm^2), xylem area (mm^2), phloem area (mm^2) and stem area (mm^2). The pith, xylem, phloem and stem areas were calculated by measuring the respective radii. Number of fibre cells in the stem were calculated by counting the number of fibre cells present in 1 mm^2 area first and then calculating the number based on the plant's total stem area. Similarly, total fibre area in the stem was calculated by tracing the fibre bundle area present in 1 mm^2 area first and then calculating total area based on the plant's stem area. The average area of individual fibre cells (μm^2) was calculated by taking the average area of five representative fibre cells. A photomicrograph of the flax stem of variety Viking with labelling of different anatomical segments is depicted in Figure 4.2.

Fibre area to stem area ratio, fibre area to phloem area ratio, fibre area to xylem area ratio, xylem area to stem area ratio and pith area to stem area ratio were calculated by dividing the respective areas from each other. This aspect is important as a greater proportion of fibre enriched stem areas as compared to other stem areas is preferred for both bio-composites, pulp and paper making industry.

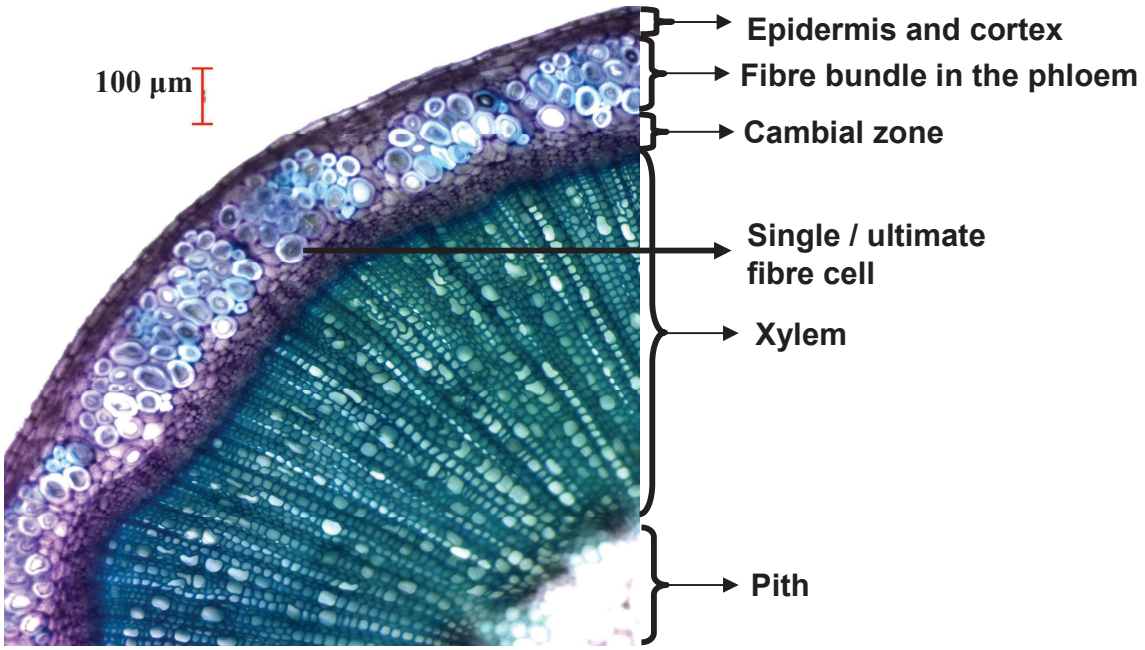


Figure 4.2. Photomicrograph of flax stem (Viking) at physiological maturity stage with labeling of different anatomical regions.

Data analysis

All statistical analyses were conducted in SAS (SAS Institute Inc., 1985 and 2005). Data were subjected to test for normality and homogeneity of error variance. Since many of the traits were heterogeneous for error variance, the data were analyzed using PROC MIXED method in SAS. REPEATED statements in SAS function were used wherever required to model the variances.

Flax genotypes were grouped into different classes: fibre flax, high fibre, low fibre and dual purpose flax and differences between the groups were explored by linear CONTRAST using the GLM method. The following square - root transformations were performed in order to homogenize error variances for the contrast analysis:

Data from the seedling stage was transformed for cortex width, total fibre area in the stem, xylem area, phloem area and fibre to xylem area ratio data for the Kernen Farm study, whereas cambial zone width, average area of single fibre cells, pith area, phloem area, fibre to xylem area ratio and xylem to stem area ratio data were transformed for the growth chamber study. At the green capsule stage; cortex width, fibre to stem area ratio and fibre to xylem area

ratio were transformed for the Kernen Farm study, while average area of single fibre cell, stem area, total fibre area, xylem area, phloem area, fibre to phloem area ratio and xylem to stem area ratio data were transformed for the growth chamber study. Similarly, at the physiological maturity stage, the average area of single fibre cells, pith area, xylem area, phloem area and fibre to phloem area ratio data were transformed for the Kernen Farm study, whereas cortex width, cambial zone width, average area of single fibre cells, total fibre area in the stem, phloem area, fibre to stem area ratio and fibre to xylem area ratio data were transformed for the growth chamber study.

Pearson correlation analysis was performed using the SAS procedure CORR for different anatomical traits at all three growth stages separately for both the Kernen Farm and growth chamber studies. The parents and the check Hermes were excluded from the correlation study as they could influence the correlation value due to the diverse nature of their stem fibre characteristics. Correlation analysis was conducted between anatomical data and 2006 multi-location field trial data (Chapter 3- for plant height and fibre concentration as estimated by the NIR instrument). Regression analysis was also performed using SAS for specific traits having significant correlations.

Results

Flax stem anatomy

The flax plants at both the Kernen Farm and growth chambers exhibited characteristic growth stages. After germination and emergence, the stem underwent a period of rapid growth, with elongation of the stem from 20 cm to 70 - 80 cm over a period of two weeks as previously noted by Gorshkova et al. (1996). Vertical growth slowed upon floral budding and sequential phases of flowering. Green capsule and physiological maturity of the flax seeds occurred over the span of six weeks. With maturity, there was a gradual increase in the stem diameter of the flax plants along with other anatomical tissues.

Sectioned flax stems from all 14 genotypes at the seedling, green capsule and physiological maturity stages exhibited fundamental histological similarities, including secondary growth by the vascular cambium. The flax stem contained the following tissues at all

the growth stages: epidermis; cortex; phloem; bast fibres; cambium; xylem and pith with a central cavity (Figure 4.2).

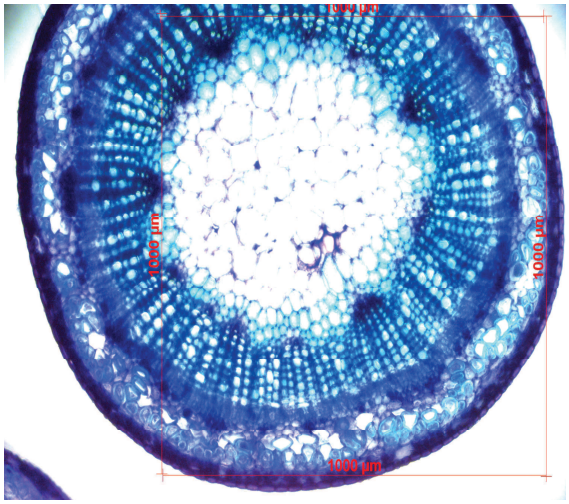
At maturity, the high fibre flax genotypes (Viking, Hermes, H341, H350, H337) had wider fibre bundles forming a continuous layer of fibre cells encircling the xylem, whereas the fibre bundles in the low fibre flax genotypes (E1747, L260, L267, L271) were smaller and more discontinuous from each other leaving gaps between each fibre bundle (Figure 4.3 and Figure 4.4).

Analysis of stem sections

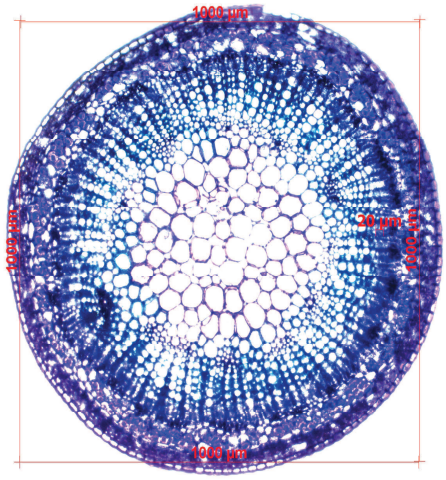
There was a wide range of variation across genotypes for all the measured traits (Table 4.2.1 and Table 4.2.2). A list of means of all the anatomical measurements of the genotypes is listed in Appendix B.

Analysis of variance was calculated for each individual trait along with their interaction with location, growth stage and genotype (Table 4.2.1 and Table 4.2.2). Genotypes were significantly different from each other for all traits except stem area, pith area, xylem area and phloem area. Stage effect was significantly different for all traits except phloem area. Environmental effects were significant at $p < 0.01$ for cortex width, average area of single fibre cells, total fibre area in the stem, fibre to stem area ratio, fibre to phloem area ratio and fibre to xylem area ratio.

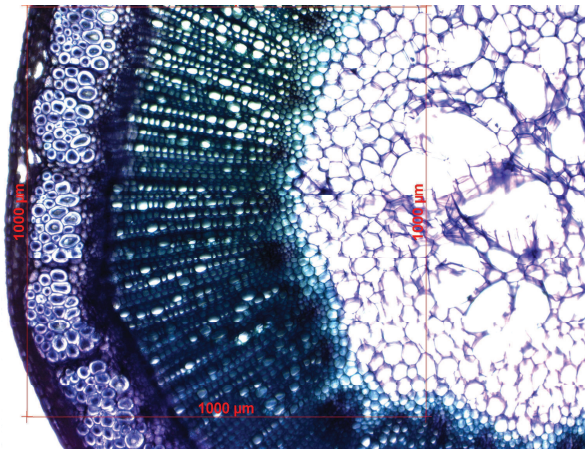
The genotype × stage interaction was highly significant ($p < 0.01$) for cortex width, number of fibre cells, total fibre area in the stem, fibre to phloem area ratio, xylem to stem area ratio and pith to stem area ratio. Genotype × environment interaction was highly significant ($p < 0.01$) in fibre to stem area ratio, fibre to xylem area ratio, xylem to stem area ratio and pith to stem area ratio. Stage × environment interactions were highly significant ($p < 0.01$) for epidermis width, cortex width, cambial zone width, average area of single fibre cells, number of fibre cells, fibre to phloem area ratio, xylem to stem area ratio and pith to stem area ratio. Three-way interaction between stage × environment × genotype was highly significant ($p < 0.01$) for epidermis width, fibre to stem area ratio, fibre to xylem area ratio, xylem to stem area ratio and pith to stem area ratio.



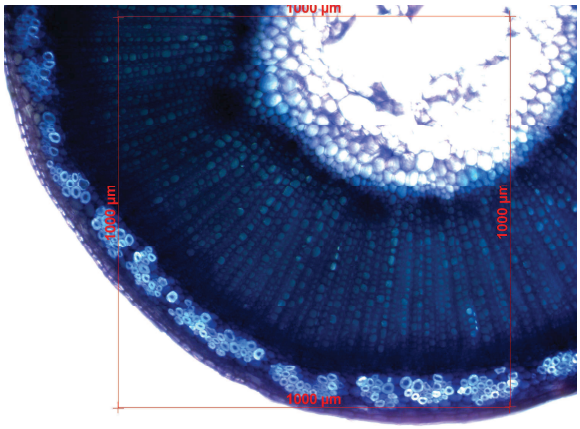
Viking (seedling stage)



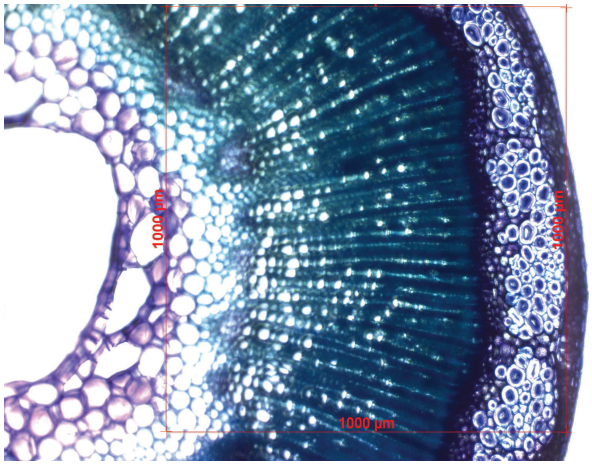
E1747 (seedling stage)



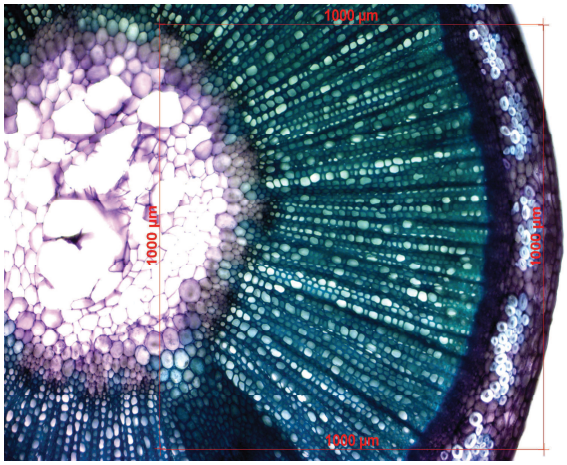
Viking (Green capsule stage)



E1747 (Green capsule stage)

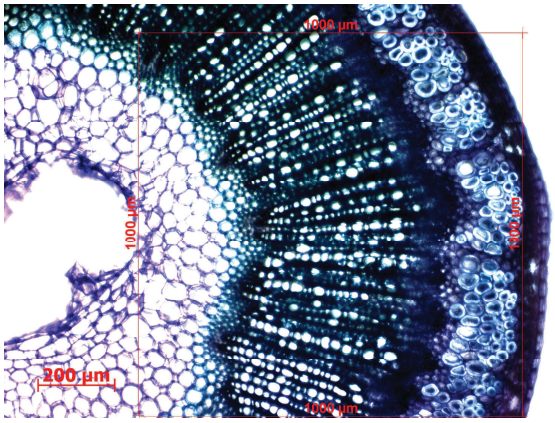


Viking (Physiological maturity stage)

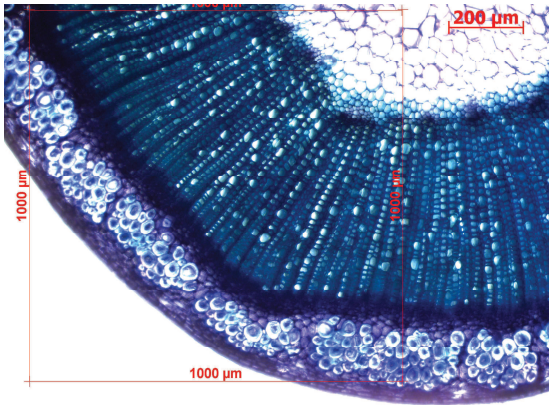


E1747 (Physiological maturity stage)

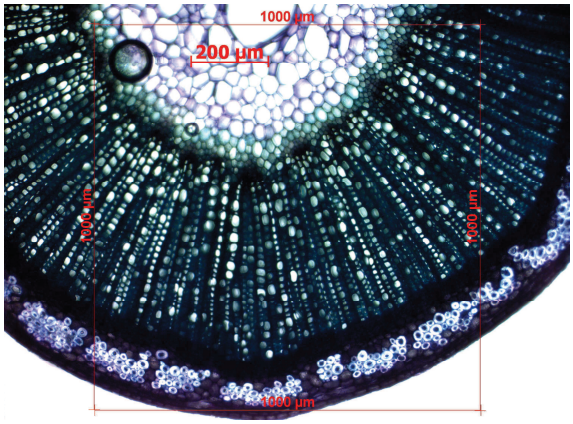
Figure 4.3. Comparative stem sections of the two parents (fibre flax type: Viking and oilseed flax type: E1747) at different growth stages at the Kernen Research Farm in 2007.



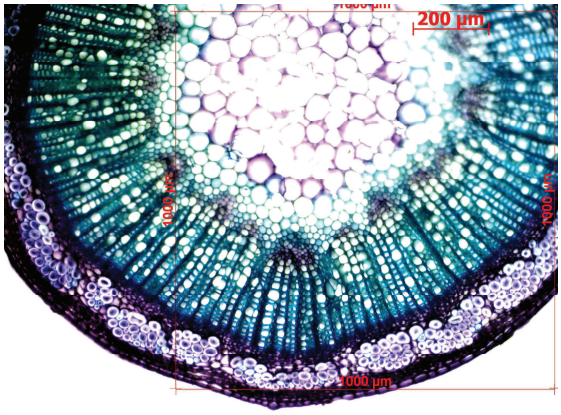
Fibre flax check (**Hermes**)



High fibre RIL (**H341**)



Low fibre RIL (**L260**)



Dual purpose RIL (**D319**)

Figure 4.4. Comparative stem cross-sections of fibre flax check (Hermes) and diverse RILs at the green capsule stage in the 2007 growth chamber study.

Table 4.2.1. F-values from the analysis of variance for anatomical traits and their interaction with different stages, environments and genotypes.

Source of variation	d.f.	Epiw	Cortw	Camw	Avg.fc area	No. fibre	Stem area	Tot. fibre area
Genotype	13	4.95 ***	9.64***	9.80 **	38.69***	12.85***	22.76	21.45 ***
Stage	2	5.48**	45.06***	93.59 ***	39.75 ***	482.97***	684.32*	124.00***
Environment	1	1.26	20.42***	6.41*	9.21**	1.48	1.91	17.01***
Genotype × Stage	26	0.94	2.58**	3.51*	3.33*	7.43**	12.60	4.87***
Genotype × Environment	13	0.92	1.80*	1.98	1.75	2.29	1.56	1.80*
Stage × Environment	2	14.28***	39.07***	12.95***	5.89**	10.10**	10.16	3.24
Stage × Environment × Genotype	26	1.88**	1.36	2.13	1.35	3.43*	1.03	1.52

*significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$.
The anatomical regions are denoted by: Epidermis width (Epiw), cortex width (Cortw), cambial zone width (Camw), average area of single fibre cells (Avg.fc area), number of fibre cells (No. fibre), total fibre area in the stem (Tot.fibre area).

Table 4.2.2. F-values from the analysis of variance for anatomical traits and their interaction with different stages, environments and genotypes.

Source of variation	d.f.	Pith area	Xylem area	Phloem area	Fibre: Stem	Fibre: Phloem	Fibre : Xylem	Xylem : Stem	Pith : Stem
Genotype	13	21.19	18.56	19.18	22.29***	7.43***	21.21***	5.50***	5.00**
Stage	2	521.42*	880.40*	148.32	9.51**	33.69***	90.63***	669.94***	5.64**
Environment	1	17.40	0.05	1.98	54.30***	20.05***	35.82***	0.16	0.03
Genotype × Stage	26	8.96	13.58	5.81	2.94*	2.89**	1.87	2.64**	4.24**
Genotype × Environment	13	7.10	1.84	2.70	3.78**	2.69*	4.44**	5.24***	3.56**
Stage × Environment	2	7.10	12.81	17.84	2.01	10.99***	0.37	35.90***	47.15***
Stage × Environment × Genotype	26	5.42	1.56	2.44	3.55**	2.60*	3.04**	4.04***	4.75**

*significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$.
The anatomical regions are denoted by: fibre to stem area ratio (Fibre : Stem), fibre to phloem area ratio (Fibre: Phloem), fibre to xylem area ratio (Fibre : Xylem), xylem to stem area ratio (Xylem : Stem) and pith to stem area ratio (Pith: Stem).

Comparative analysis of fibre flax vs. oilseed flax genotypes

Differences between fibre flax genotypes and oilseed flax genotypes were explored by linear contrasts. Viking and Hermes together were considered as the fibre flax group; E1747, L271, L260 and L267 were considered as low fibre group and H341, H350, H337 were considered as high fibre group for the contrast analysis.

There were highly significant differences ($p < 0.01$) observed between the fibre flax group and the low fibre group for average area of single fibre cells, total fibre area and stem area at all the three growth stages during both the Kernen Farm and growth chamber studies (Table 4.3). From green capsule stage onwards, significant ($p < 0.01$) differences were also found between the fibre flax group and the low fibre group for fibre to stem area ratio, number of fibre cells, xylem area and phloem area for both the Kernen Farm and growth chamber studies.

Interestingly, there were similar differences between the fibre flax and the high fibre group for average area of fibre cells, phloem area, pith area, xylem area, stem area for green capsule and physiological maturity stage at both the Kernen Farm and growth chamber. However, the magnitude of differences was less as compared to the differences between fibre flax and low fibre group (Table 4.3).

Comparison between the parents and the recombinant inbred lines

Viking vs. E1747

In both the Kernen Farm and growth chamber experiments, Viking had significantly larger average area of single fibre cells as compared to E1747 ($p < 0.01$) at all plant growth stages except for the seedling stage in the growth chamber study. Viking had significantly higher total fibre area as compared to E1747 in both the Kernen Farm and growth chamber studies at two different growth stages ($p < 0.01$). Fibre to stem area ratio and fibre to xylem area ratio were significantly higher in Viking as compared to E1747 ($p < 0.01$) at the green capsule and physiological maturity stage in the growth chamber study (Tables 4.4.1 - 4.4.3). Pictures of the two parents growing at the Kernen Farm and the growth chamber are depicted in Figures 4.1.1, 4.1.2 and 4.3.

Table 4.3. F- values for the contrast analysis between anatomical traits of fibre flax genotypes and low and high fibre genotypes at the Kernen Farm and growth chamber locations. Fibre flax group: Viking, Hermes; low fibre group: E1747, L271, L260, L267; high fibre group: H341, H350, H337. Growth stages: seedling (Seed); green capsule (GC); physiological maturity (Mat).

Kernen Farm	**Epiw**			**Cortw**			**Camw**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Fibre flax vs. low fibre	24.77***	0.13	3.42	12.23**	0.20	2.34	10.11**	0.09	1.89
Fibre flax vs. high fibre	49.82***	0.34	0.01	0.68	3.87	0.11	13.47**	0.15	2.90
	Avg. fc area			**No. fibre**			**Stem area**		
Fibre flax vs. low fibre	50.09***	48.59***	47.08***	2.40	10.55**	27.64***	15.88**	23.82***	59***
Fibre flax vs. high fibre	27.19***	5.31*	9.69**	2.63	5.00*	17.92**	3.47	7.21*	44.26***
	Tot.fibre area			**Pith area**			**Xylem area**		
Fibre flax vs. low fibre	35.15***	17.88**	71.07***	4.37*	31.74***	51.02***	6.43*	21.32***	35.90***
Fibre flax vs. high fibre	10.28**	1.91	49.23***	0.10	9.91**	50.87***	0.26	4.39*	21.76**
	Phloem area			**Fibre: Stem**			**Fibre: Phloem**		
Fibre flax vs. low fibre	5.98*	22.66***	50.56***	34.09***	8.21**	30.43***	17.15**	2.40	4.54*
Fibre flax vs. high fibre	8.05**	5.28*	40.24***	13.30**	0.01	11.75**	0.27	0.20	0.28
	Fibre: Xylem			**Xylem: Stem**			**Pith: Stem**		
Fibre flax vs. low fibre	32.23***	6.18*	20.04***	3.76	1.40	0.86	0.47	0.51	0.01
Fibre flax vs. high fibre	14.45**	0.05	9.59**	2.90	2.01	0.39	0.95	0.06	0.39
Growth Chamber	**Epiw**			**Cortw**			**Camw**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Fibre flax vs. low fibre	0.01	2.87	8.18**	0.79	3.59	0.01	7.71**	3.97	7.56*
Fibre flax vs. high fibre	1.14	2.21	6.07*	0.91	3.2	0.05	9.06**	0.58	1.76
	Avg.fc area			**No. fibre**			**Stem area**		
Fibre flax vs. low fibre	32.06***	101.63***	84.21***	0.04	28.73***	21.51***	11.15**	53.34***	57.05***
Fibre flax vs. high fibre	1.99	31.25***	28.80***	4.15	9.54**	1.61	0.56	24.07***	47.58***
	Tot.fibre area			**Pith area**			**Xylem area**		
Fibre flax vs. low fibre	11.91**	103.16***	98.76***	2.00	93.90***	2.87	4.40*	26.74***	47.24***
Fibre flax vs. high fibre	0.34	36.84***	37.61***	3.11	42.16***	8.12**	3.9	28.58***	48.15***
	Phloem area			**Fibre: Stem**			**Fibre: Phloem**		
Fibre flax vs. low fibre	2.52	58.31***	75.95***	1.80	59.43***	92.64***	0.02	1.74	36.41***
Fibre flax vs. high fibre	0.42	25.09***	36.02***	0.01	13.83**	15.20**	0.02	0.07	6.98*
	Fibre: Xylem			**Xylem: Stem**			**Pith: Stem**		
Fibre flax vs. low fibre	3.40	70.13***	75.47***	0.58	12.57**	0.30	2.17	6.75*	52.64***
Fibre flax vs. high fibre	1.60	6.16*	7.74**	1.48	3.69	3.63	1.80	0.82	12.42**

*significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$.

← **Table 4.3.** The anatomical regions are denoted by: Epidermis width (Epiw), cortex width (Cortw), cambial zone width (Camw), average area of single fibre cell (Avg.fc area), number of fibre cells (No. fibre), total fibre area in the stem (Tot.fibre area), fibre to stem area ratio (Fibre : Stem), fibre to phloem area ratio (Fibre: Phloem), fibre to xylem area ratio (Fibre : Xylem), xylem to stem area ratio (Xylem : Stem) and pith to stem area ratio (Pith: Stem).

High fibre RILs (H341, H350 and H337) vs. Low fibre RILs (L271, L260, L267)

The high fibre RILs had significantly greater total fibre area ($p < 0.05$ to $p < 0.0001$) at all the growth stages and at both the Kernen Farm and growth chamber studies (Table 4.4.2). From green capsule stage onwards, fibre to stem area ratio and average area of fibre cell were significantly higher ($p < 0.01$) in high fibre RILs as compared to the low fibre RILs in both the Kernen Farm and growth chamber studies (Tables 4.4.1 and 4.4.2). A list of all other significant differences between the two groups is depicted in Tables 4.4.1- 4.4.3.

High / low fibre RILs vs. Dual purpose RILs (D311, D273, D319)

Cortex width was the only anatomical trait that consistently was significantly greater in dual purpose genotypes as compared to the high or low fibre RIL group at both the Kernen Farm and growth chamber studies from the green capsule stage onwards (at $p < 0.05$ level to $p < 0.0001$) (Table 4.4.1). In the growth chamber study, the dual purpose RILs had significantly lower ($p < 0.01$) fibre to stem area ratio and fibre to xylem area ratio (except at seedling stage) as compared to the high fibre RILs (Tables 4.4.2 and 4.4.3). There were also some significant differences between dual purpose lines and the high / low fibre RILs; however these differences were inconsistent over either stages or locations (Tables 4.4.1 - 4.4.3).

Short (S268) vs. tall (T284) RIL

Fibre to xylem area ratio was significantly higher ($p < 0.05$ to $p < 0.0001$) in the short RIL as compared to the tall RIL from the green capsule stage onwards at both the Kernen Farm and growth chamber studies (Table 4.4.3). Fibre to stem area ratio and xylem to stem area ratio were also significantly higher ($p < 0.05$ to $p < 0.0001$) in the short RIL as compared to the tall RIL from green capsule stage onwards, but only in the growth chamber experiment (Tables 4.4.2 and 4.4.3). Total fibre area was significantly higher (ranging from $p < 0.05$ to $p < 0.01$) in the

short RIL as compared the tall RIL but only in the growth chamber study at the seedling and green capsule stages (Table 4.4.2).

Table 4.4.1. F- values for the contrast analysis between anatomical traits of parents (Viking and E1747) and diverse RILs at field and growth chamber studies. Low fibre group: L271, L260, L267; high fibre group: H341, H350, H337; dual purpose group: D319, D311, D273; Short RIL: S 268; Tall RIL: T284. Growth stages: seedling (Seed); green capsule (GC); physiological maturity (Mat).

Kernen Farm	**Epiw**			**Cortw**			**Camw**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Viking vs. E1747	10.97**	6.84*	5.51*	6.19*	0.23	0.01	1.90	0.47	1.45
High fibre vs. Low fibre RILs	7.14*	4.48*	1.84	7.51*	6.98*	5.79*	0.02	0.28	0.35
High fibre vs. Dual purpose RILs	4.27*	1.70	0.01	0.01	24.87***	28.62***	1.97	2.10	0.01
Low fibre vs. Dual purpose RILs	0.37	0.66	1.58	8.17**	5.50*	8.67**	2.40	0.85	0.25
Short vs. Tall RILs	1.46	0.22	0.02	0.06	0.67	6.52*	0.50	0.00	0.05
Growth chamber	**Epiw**			**Cortw**			**Camw**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Viking vs. E1747	1.54	1.51	2.04	0.31	0.70	0.03	2.16	0.00	1.56
High fibre vs. Low fibre RILs	0.88	0.63	1.48	2.56	0.00	0.22	0.13	3.58	12.22**
High fibre vs. Dual purpose RILs	0.14	2.33	0.54	0.51	30.18***	17.69*	4.02	0.01	0.89
Low fibre vs. Dual purpose RILs	1.71	0.54	0.24	0.79	30.92***	13.99**	5.58*	3.99	19.72**
Short vs. Tall RILs	0.01	4.06	0.06	0.85	0.16	0.91	0.14	0.34	0.01
Kernen Farm	**Avg.fc area**			**No.fibre**			**Stem area**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Viking vs. E1747	8.85**	15.99**	9.23**	9.06**	0.13	0.41	16.26**	3.72	10.2**
High fibre vs. Low fibre RILs	4.12	23.99***	20.79**	8.27**	2.82	3.91	3.83	8.64**	3.08
High fibre vs. Dual purpose RILs	0.51	10.78**	0.65	0.54	0.02	3.44	0.60	2.66	1.02
Low fibre vs. Dual purpose RILs	7.53*	2.61	14.09**	13.03**	3.34	14.69**	1.39	1.71	7.63**
Short vs. Tall RILs	2.78	4.65*	0.92	0.03	0.43	1.38	0.14	0.20	0.69
Growth chamber	**Avg.fc area**			**No.fibre**			**Stem area**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Viking vs. E1747	9.58*	21.71***	15.85**	0.81	8.01*	4.80*	5.73*	10.84**	7.54*
High fibre vs. Low fibre RILs	18.08**	21.47**	16.77**	5.31*	6.18*	14.12	5.07*	8.45*	1.19
High fibre vs. Dual purpose RILs	8.22*	14.96**	10.06**	10.34**	3.65	5.01	8.72**	7.13*	0.05
Low fibre vs. Dual purpose RILs	1.92	0.59	0.85	30.48***	0.33	2.31	0.49	0.06	0.75
Short vs. Tall RILs	2.46	7.50*	0.38	1.90	0.66	4.16	2.67	0.01	2.99

*significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$.
The anatomical regions are denoted by: Epidermis width (Epiw), cortex width (Cortw), cambial zone width (Camw), average area of single fibre cells (Avg.fc area), number of fibre cells (No. fibre).

Table 4.4.2. F- values for the contrast analysis between anatomical traits of parents (Viking and E1747) and diverse RILs at field and growth chamber studies. Low fibre group: L271, L260, L267; high fibre group: H341, H350, H337; dual purpose group: D319, D311, D273; Short RIL: S 268; Tall RIL: T284. Growth stages: seedling (Seed); green capsule (GC); physiological maturity (Mat).

Kernen Farm	**Tot.fibre area**			**Pith area**			**Xylem area**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Viking vs. E1747	16.55**	1.94	11.79**	15.93**	0.88	4.14	12.17**	4.11	6.17*
High fibre vs. Low fibre RILs	7.80**	12.34**	5.16*	0.68	12.29**	0.69	3.16	10.63**	4.66*
High fibre vs. Dual purpose RILs	0.14	4.06	3.08	2.48	2.21	5.45*	1.05	4.82*	0.18
Low fibre vs. Dual purpose RILs	5.84*	2.24	16.21**	0.57	4.08	10.01**	0.56	1.13	6.67*
Short vs. Tall RILs	0.48	1.79	2.80	0.004	0.06	0.02	0.08	0.10	3.02
Growth chamber	**Tot.fibre area**			**Pith area**			**Xylem area**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Viking vs. E1747	2.49	31.44***	19.67***	4.13	39.26***	7.84**	7.66**	2.10	6.40*
High fibre vs. Low fibre RILs	7.14*	16.25**	15.74**	2.23	10.70**	4.84*	0.50	0.15	0.26
High fibre vs. Dual purpose RILs	1.20	17.69**	9.99**	3.08	4.66*	1.52	0.91	0.43	0.12
Low fibre vs. Dual purpose RILs	14.18**	0.03	0.65	10.55**	1.24	0.93	2.75	0.07	0.74
Short vs. Tall RILs	8.41**	8.37*	3.68	10.60**	1.64	2.37	2.82	1.36	11.86**
Kernen Farm	**Phloem area**			**Fibre: Stem**			**Fibre: Phloem**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Viking vs. E1747	3.54	7.21*	10.47**	5.80*	0.44	2.03	6.10*	0.31	0.52
High fibre vs. Low fibre RILs	0.20	9.10**	1.06	6.03*	12.67**	10.05**	24.11***	6.39*	9.28*
High fibre vs. Dual purpose RILs	3.01	2.07	0.62	0.07	5.55*	3.42	11.63**	1.88	3.00
Low fibre vs. Dual purpose RILs	1.67	2.49	3.31	7.42*	1.45	25.21***	2.25	1.34	22.82***
Short vs. Tall RILs	0.65	3.92	0.63	3.33	2.60	16.45**	4.65*	0.20	2.78
Growth chamber	**Phloem area**			**Fibre: Stem**			**Fibre: Phloem**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Viking vs. E1747	0.32	14.79**	6.31*	0.09	23.14***	22.74***	0.01	1.37	16.97**
High fibre vs. Low fibre RILs	6.72*	8.48**	11.02**	2.09	12.37**	33.78***	0.40	0.53	10.10**
High fibre vs. Dual purpose RILs	0.28	8.13**	11.65**	11.24**	16.72***	25.47***	0.12	0.61	0.08
Low fibre vs. Dual purpose RILs	4.26*	0.01	0.01	23.04***	0.33	0.58	0.08	0.01	2.02
Short vs. Tall RILs	0.03	2.85	2.89	3.30	18.38**	14.75**	0.74	0.01	1.31

*significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$.
The anatomical regions are denoted by: total fibre area in the stem (Tot.fibre area), fibre to stem area ratio (Fibre : Stem), fibre to phloem area ratio (Fibre: Phloem).

Table 4.4.3. F- values for the contrast analysis between anatomical traits of parents (Viking and E1747) and diverse RILs at the Kernen farm and growth chamber studies. Low fibre group: L271, L260, L267; high fibre group: H341, H350, H337; dual purpose group: D319, D311, D273; Short RIL: S 268; Tall RIL: T284. Growth stages: seedling (Seed); green capsule (GC); physiological maturity (Mat).

Kernen Farm	**Fibre: Xylem**			**Xylem: Stem**			**Pith: Stem**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Viking vs. E1747	3.46	0.19	0.69	0.01	0.73	0.47	3.12	1.26	0.64
High fibre vs. Low fibre RILs	4.98	7.43*	4.29*	0.27	9.36**	4.49*	0.78	0.68	0.37
High fibre vs. Dual purpose RILs	0.12	2.27	4.31*	0.12	11.33**	1.93	1.05	0.07	0.63
Low fibre vs. Dual purpose RILs	6.64*	1.49	17.21**	0.76	0.09	0.53	3.65	1.21	0.03
Short vs. Tall RILs	1.79	4.46*	21.74***	0.001	9.41	10.24	0.29	0.1	0.59
Growth chamber	**Fibre: Xylem**			**Xylem: Stem**			**Pith: Stem**		
	Seed	**GC**	**Mat**	**Seed**	**GC**	**Mat**	**Seed**	**GC**	**Mat**
Viking vs. E1747	0.01	30.22***	19.03**	0.42	10.61**	0.02	0.31	8.71*	0.87
High fibre vs. Low fibre RILs	11.84**	30.67***	34.34***	6.42*	28.87***	0.59	17.17**	1.98	41.70***
High fibre vs. Dual purpose RILs	2.10	30.01***	28.58***	1.38	17.71**	1.35	0.02	0.06	7.05*
Low fibre vs. Dual purpose RILs	23.92***	0.01	0.26	1.85	1.36	0.16	18.25**	2.75	14.46***
Short vs. Tall RILs	3.54	25.64***	27.60***	0.11	6.16*	22.39***	7.55*	2.4	0.14

*significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$.
The anatomical regions are denoted by: Fibre to xylem area ratio (Fibre : Xylem), xylem to stem area ratio (Xylem : Stem) and pith to stem area ratio (Pith: Stem).

Correlation analysis

Pearson correlation analysis was conducted for all the three stages of plant growth as well as for both the Kernen Farm (r_f) and growth chamber (r_p) studies to test if relationships existed among the anatomical traits. Correlation analysis revealed significant positive linear relationships between various variables.

Seedling stage

The highest positive correlation was observed between total fibre area and fibre to stem area ratio for both growth chamber ($r_p = 0.86$, $p < 0.0001$) as well as in the Kernen Farm ($r_f = 0.81$, $p < 0.0001$) studies. Fibre to stem area ratio was also positively correlated with number of fibre cells ($r_f = 0.43$, $p < 0.05$; $r_p = 0.75$, $p < 0.0001$) (Table 4.5.1).

The other significant correlations were between phloem area and stem area ($r_f = 0.73$, $p < 0.0001$, $r_p = 0.73$, $p < 0.0001$) and between total fibre area and number of fibre cells ($r_f = 0.71$, $r_p = 0.69$, $p < 0.0001$) in both the Kernen Farm and growth chamber studies. Lists of all other correlations are depicted in Table 4.5.1.

Green capsule stage

The highest correlation was observed between fibre to stem area ratio and total fibre area ($r_f = 0.85$ and $r_p = 0.89$, $p < 0.0001$). Fibre to stem area ratio was also significantly correlated with average area of single fibre cells ($r_f = 0.59$, $p < 0.01$ and $r_p = 0.61$, $p < 0.0001$), number of fibre cells ($r_f = 0.48$, $p < 0.01$ and $r_p = 0.49$, $p < 0.01$), phloem area ($r_f = 0.57$, $p < 0.01$ and $r_p = 0.71$, $p < 0.0001$) and pith area ($r_f = 0.48$, $p < 0.01$ and $r_p = 0.42$, $p < 0.05$) (Table 4.5.2).

Total fibre area was positively correlated with phloem area ($r_f = 0.84$ and $r_p = 0.82$, $p < 0.0001$), average area of single fibre cell ($r_f = 0.71$ and $r_p = 0.79$, $p < 0.0001$), stem area ($r_f = 0.81$ and $r_p = 0.70$, $p < 0.0001$), pith area ($r_f = 0.74$ and $r_p = 0.65$, $p < 0.0001$), number of fibre cells ($r_f = 0.79$, $p < 0.0001$ and $r_p = 0.56$, $p < 0.01$) and xylem area ($r_f = 0.77$, $p < 0.0001$ and $r_p = 0.54$, $p < 0.01$) in both the Kernen Farm and growth chamber studies (Table 4.5.2).

The average area of single fibre cells was significantly correlated with stem area, number of fibre cells, pith area, phloem area and cambial zone width at both the locations ranging from

$p < 0.05$ to $p < 0.0001$ levels of significance. Number of fibre cells was significantly correlated with stem area, xylem area, and phloem area in both the Kernen Farm and growth chamber studies from $p < 0.05$ to $p < 0.0001$ levels of significance. Stem area was significant ($p < 0.0001$) and positively correlated with xylem area ($r_f = 0.98$ and $r_p = 0.67$). A few negative correlations were also observed between different anatomical traits but they were not significant in one or both the locations. A list of all other correlations is depicted in Table 4.5.2.

Physiological maturity stage

The highest positive correlation was observed between stem area and xylem area ($r_f = 0.96$ and $r_p = 0.91$, $p < 0.0001$) followed by fibre to stem area ratio and total fibre area ($r_f = 0.77$ and $r_p = 0.92$, $p < 0.0001$). The fibre to stem area ratio was also significantly correlated with the number of fibre cells ($r_f = 0.77$ and $r_p = 0.74$, $p < 0.0001$), phloem area ($r_f = 0.48$, $p < 0.01$ and $r_p = 0.66$, $p < 0.0001$) and average area of single fibre cells ($r_f = 0.40$, $p < 0.05$ and $r_p = 0.72$, $p < 0.0001$) in both the Kernen Farm and growth chamber studies**.** The other correlations between the anatomical traits were similar to that observed in the green capsule stage and are depicted in Table 4.5.3.

A list of all other correlations between ratios of the anatomical traits such as fibre to phloem area ratio, fibre to xylem area ratio, xylem to stem area ratio and pith to stem area ratio are depicted in Appendix B, Tables B.1- B.3.

Table 4.5.1. Pearson correlation coefficients between anatomical and multi-location field traits (plant height and NIR fibre content) at the seedling stage.

Anatomical traits		Cortw	Camw	Avg.fc area	No.fibre	Stem area	Tot.fibre area	Pith area	Xylem area	Phloem area	Fibre: Stem	Plant ht	NIR Fibre
Epiw	f	-0.07	0.03	-0.09	-0.05	-0.04	-0.40	-0.01	-0.08	0.20	-0.03	-0.17	-0.36
	p	-0.07	0.34	0.33	-0.33	0.31	-0.20	0.57**	0.59**	0.00	-0.30	0.08	-0.16
Cortw	f		0.20	0.20	0.41*	0.47**	0.43*	0.08	0.36*	0.32	0.26	0.13	0.43*
	p		-0.09	0.01	0.20	-0.14	0.07	-0.07	0.12	-0.16	0.91	0.11	0.26
Camw	f			-0.08	0.20	0.30	0.13	0.42*	0.31	0.18	-0.06	-0.26	-0.16
	p			0.30	-0.35	0.17	-0.01	0.504**	0.30	0.12	-0.13	0.07	-0.13
Avg.fc area	f				0.08	0.15	0.38*	0.18	0.04	0.15	0.51**	0.30	0.45**
	p				-0.22	0.67***	0.25	0.53**	0.50**	0.50**	-0.08	0.23	0.51**
No. fibre	f					0.72	0.71***	0.43*	0.70***	0.64***	0.43*	0.01	0.40*
	p					-0.27	0.69***	-0.29	-0.22	0.00	0.75***	-0.19	0.26
Stem area	f						0.84***	0.49**	0.85***	0.73***	0.38*	0.05	0.31
	p						0.10	0.58**	0.33	0.73***	-0.41*	0.05	0.32
Tot.fibre area	f							0.40*	0.74***	0.65***	0.81***	0.09	0.48**
	p							0.10	0.11	0.22	0.86***	-0.06	0.48**
Pith area	f								0.43*	0.31	0.21	-0.03	0.05
	p								0.78***	0.26	-0.22	-0.18	-0.10
Xylem area	f									0.53**	0.362*	0.02	0.22
	p									-0.03	0.08	-0.06	-0.07
Phloem area	f										0.31*	0.06	-0.01
	p										-0.14	0.11	0.37*
Fibre: Stem	f											0.09	0.52**
	p											-0.05	0.27

p= growth chamber, f= Kernen Farm. *significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$.
The anatomical regions are denoted by: Epidermis width (Epiw), cortex width (Cortw), cambial zone width (Camw), average area of single fibre cells (Avg.fc area), number of fibre cells (No. fibre), total fibre area in the stem (Tot.fibre area), fibre to stem area ratio (Fibre : Stem), plant height (Plant ht), NIR fibre % (NIR fibre).

Table 4.5.2. Pearson correlation coefficients between anatomical and multi-location field traits (plant height and NIR fibre content) at the green capsule stage.

		Cortw	Camw	Avg.fc area	No. fibre	Stem area	Tot.fibre area	Pith area	Xylem area	Phloem area	Fibre: Stem	Plant ht	NIR Fibre
Epiw	f	-0.48**	0.40**	0.69***	0.26	0.42*	0.44*	0.26	0.41*	0.47*	0.35*	0.28	0.40*
	p	-0.23	0.15	0.07	0.05	0.34	0.20	0.21	0.40*	0.36*	0.13	0.29	0.05
Cortw	f		-0.28	-0.50**	0.05	-0.25	-0.28	-0.21	-0.31	-0.24	-0.24	-0.26	-0.21
	p		0.08	-0.32	0.14	-0.24	-0.17	-0.15	-0.12	-0.17	-0.13	-0.07	0.13
Camw	f			0.38*	0.53**	0.68***	0.57**	0.42*	0.67***	0.56**	0.33	0.07	0.10
	p			0.388*	0.14	0.45**	0.44*	0.40*	0.49**	0.49**	0.30	0.39	0.32
Avg.fc area	f				0.38*	0.60**	0.71***	0.44**	0.57**	0.74***	0.59**	0.12	0.63***
	p				0.45**	0.69***	0.79***	0.54**	0.32	0.66***	0.61***	0.11	0.61***
No. fibre	f					0.85***	0.79***	0.82***	0.79***	0.67***	0.48**	0.08	0.37*
	p					0.46**	0.56**	0.18	0.45**	0.49**	0.49**	-0.07	0.36*
Stem area	f						0.81***	0.81***	0.98***	0.83***	0.42*	0.31	0.46**
	p						0.70***	0.71***	0.67***	0.66***	0.33	0.29	0.40
Tot.fibre area	f							0.74***	0.77***	0.84***	0.85***	0.04	0.55**
	p							0.65***	0.54**	0.82***	0.89***	0.12	0.47**
Pith area	f								0.81***	0.53**	0.48**	0.19	0.53**
	p								0.54**	0.52**	0.42*	0.02	0.40*
Xylem area	f									0.75***	0.38*	0.37*	0.46**
	p									0.45**	0.30	0.41*	-0.01
Phloem area	f										0.57**	0.16	0.51**
	p										0.71***	0.16	0.41*
Fibre: Stem	f											-0.15	0.46**
	p											-0.02	0.38*

p= growth chamber, f= Kernen Farm. *significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$.
The anatomical regions are denoted by: Epidermis width (Epiw), cortex width (Cortw), cambial zone width (Camw), average area of single fibre cells (Avg.fc area), number of fibre cells (No. fibre), total fibre area in the stem (Tot.fibre area), fibre to stem area ratio (Fibre : Stem, plant height (Plant ht), NIR fibre % (NIR fibre).

Table 4.5.3. Pearson correlation coefficients between anatomical and multi-location field traits (plant height and NIR fibre content) at the physiological maturity stage.

Anatomical traits		Cortw	Camw	Avg.fc area	No.fibre	Stem area	Tot.fibre area	Pith area	Xylem area	Phloem area	Fibre: Stem	Plant ht	NIR Fibre
Epiw	f	-0.02	0.56**	0.02	0.11	0.16	0.10	0.13	0.10	0.05	-0.03	-0.10	-0.17
	p	-0.01	0.37*	0.08	0.06	0.19	0.15	-0.10	0.15	0.29	0.10	0.00	0.19
Cortw	f		-0.20	-0.19	0.34*	-0.06	0.16	0.10	-0.22	0.05	0.30	-0.33	-0.12
	p		0.33	-0.13	0.30	0.09	-0.06	0.21	0.02	-0.13	-0.10	-0.13	0.09
Camw	f			0.27	0.05	0.24	0.20	0.12	0.22	0.00	0.07	0.22	0.17
	p			0.31	0.28	0.46**	0.36*	0.12	0.39*	0.33	0.24	0.35*	0.54**
Avg.fc area	f				0.22	0.63**	0.58**	0.46	0.60**	0.54**	0.40*	0.28	0.55**
	p				0.53**	0.67***	0.86***	0.43	0.43	0.83***	0.72***	0.38*	0.50**
No. fibre	f					0.56**	0.84***	0.56**	0.42**	0.61**	0.77***	0.07	0.41*
	p					0.35*	0.71***	0.17	0.10	0.53**	0.74***	-0.08	0.55**
Stem area	f						0.81***	0.76***	0.96***	0.79***	0.28	0.45**	0.27
	p						0.61**	0.79***	0.91***	0.74***	0.27	0.55**	0.15
Tot.fibre area	f							0.71***	0.69***	0.79***	0.77***	0.14	0.45**
	p							0.40*	0.32	0.85***	0.92***	0.16	0.51**
Pith area	f								0.69**	0.53**	0.34	0.34	0.20
	p								0.66***	0.51**	0.10	0.17	-0.24
Xylem area	f									0.69***	0.13	0.54**	0.26
	p									0.44*	-0.01	0.69***	0.04
Phloem area	f										0.48**	0.04	0.24
	p										0.66***	0.25	0.36*
Fibre: Stem	f											-0.18	0.54**
	p											-0.06	0.59**

p= growth chamber, f= Kernen Farm. *significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$.
The anatomical regions are denoted by: Epidermis width (Epiw), cortex width (Cortw), cambial zone width (Camw), average area of single fibre cells (Avg.fc area), number of fibre cells (No. fibre), total fibre area in the stem (Tot.fibre area), fibre to stem area ratio (Fibre : Stem), Plant height (Plant ht), NIR fibre % (NIR fibre).

Correlation analysis between anatomical traits and multi-location field traits (Chapter 3)

Plant height

There were no significant correlations between plant height (from multi-location pooled field data, Chapter 3) and any anatomical traits at the seedling stage in both the Kernen Farm and growth chamber studies. At the green capsule stage, plant height was correlated with xylem area ($r_f = 0.37$ and $r_p = 0.41$, $p < 0.05$) in both the Kernen Farm and growth chamber studies. At the physiological maturity stage, plant height was significantly correlated with xylem area ($r_f = 0.54$, $p < 0.01$ and $r_p = 0.69$, $p<0.0001$) and stem area ($r_f = 0.45$ and $r_p = 0.55$, $p < 0.01$) in both the Kernen Farm and growth chamber studies.

There were no significant negative correlations between plant height and any of the anatomical traits over both the Kernen Farm and growth chamber studies. A list of all correlation between plant height and anatomical traits are depicted in Tables 4.5.1 - 4.5.3.

Fibre concentration (NIR fibre %)

Fibre concentration as estimated by NIR (from multi-location pooled field data, Chapter 3) was positively correlated with seedling stage measurements of total fibre area in the stem ($r_f = 0.48$ and $r_p = 0.48$, $p < 0.01$) and average area of single fibre cells ($r_f = 0.45$ and $r_p = 0.51$ $p < 0.01$) in both the Kernan Farm and growth chamber studies (Table 4.5.1).

At the green capsule stage, the highest correlation for NIR fibre concentration and anatomical measurements was with average area of single fibre cell ($r_f = 0.63$ and $r_p = 0.61$, $p < 0.0001$) followed by total fibre area ($r_f = 0.55$ and $r_p = 0.47$, $p < 0.01$), fibre to stem area ratio ($r_f = 0.46$, $p < 0.01$ and $r_p = 0.38$, $p < 0.05$), phloem area ($r_f = 0.51$, $p < 0.01$ and $r_p = 0.41$, $p < 0.05$), pith area ($r_f = 0.53$, $p < 0.01$ and $r_p = 0.40$, $p < 0.05$) and number of fibre cells ($r_f = 0.37$ and $r_p = 0.36$, $p < 0.05$) in both the Kernen Farm and growth chamber studies (Table 4.5.2).

Similar results were also observed at the physiological maturity stage where NIR fibre concentration had the highest correlation with anatomical measurements for fibre to stem area ratio ($r_f = 0.54$ and $r_p = 0.59$, $p < 0.01$) followed by average area of single fibre cell ($r_f = 0.55$ and $r_p = 0.50$, $p < 0.01$), total fibre area ($r_f = 0.45$ and $r_p = 0.51$, $p < 0.01$) and number of fibre cells ($r_f = 0.41$, $p < 0.01$ and $r_p = 0.55$, $p < 0.05$) (Table 4.5.3). Some negative correlations were also

found but they were non significant and inconsistent over the growth stages or locations. A list of all correlation between NIR fibre concentration and anatomical traits are depicted in Tables 4.5.1 - 4.5.3.

Discussion

The structural features detected in the stems of the 14 genotypes tested, corroborated earlier findings (Esau, 1943) in *L. usitatissimum* L. Stem diameter, cortex, phloem, fibre and xylem thickness showed a gradual increase with the progress in age. At physiological maturity, the fibre bundles became wider and more prominent with secondary cell wall thickening. The outermost fibre cells were the first to enlarge their transverse diameters and develop secondary thickening. This progression was also observed in studies using autoradiography and cellufluor staining by Gorshkova et al. (2003). The fibre cells exhibited pentagonal / hexagonal or slightly rounded shape similar to that observed by Dijon (2002). The flax stem was round or subterete, and became hollow at maturity. This result was also found by Esau (1943), who concluded that the hollowness was due to the progressive disintegration of the centrally located pith cells (Figures 4.3 and 4.4).

In the past there has been very limited research into flax anatomy with respect to differences between oilseed flax and fibre flax genotypes. In this study, highly significant differences were found between different genotypes based on anatomical traits, indicating the possibility of selection and improvement of fibre content using hybridization between the two flax types. Though some genotype x environment interaction existed, the overall ranking between high fibre and low fibre lines did not change at any growth stage and in both Kernen Farm and growth chamber studies (Appendix B), indicating the absence of cross over interaction (Gregorius and Namkoong, 1986).

Among the 14 genotypes, the fibre flax cultivars Viking and Hermes were found to have highest values for most of the anatomical traits such as average area of single fibre cells, total fibre area, fibre to stem area ratio and stem area. The genotypes with the lowest ranking for all the anatomical traits belonged to the low fibre group (L260, L267 and L271) in all the growth stages and in both the Kernen Farm and growth chamber studies.

Fibre flax vs. oilseed flax

Previous studies by van Dam et al. (1996) and Sankari (2000a) indicated that fibre obtained from oilseed flax was similar to that of fibre flax, but because of the shorter stem growth, the fibre bundles were shorter. This result was also confirmed in the present study, as the fibre bundles were smaller in cross-sectional area and were loosely packed in the low fibre group as compared to the fibre flax group, which on the other hand formed a wider and more continuous layer of fibre cells/ bundles encircling the xylem tissues (Figures 4.3 and 4.4).

Fröier (1960) reported that fibre flax cultivars had well filled fibre cells with small lumen, whereas in the oilseed flax types the fibre cells were very large and empty. Sankari (2000a) supported this observation and reported fibre cell diameter of oilseed type flax varieties ranging from 26-38 μm, which clearly exceeded that of fibre flax varieties (17-24 μm). However, in the present study, the two European fibre flax varieties, namely Viking and Hermes had larger average area of single fibre cells (19-24 μm fibre cell diameter) as compared to the oilseed genotype, E1747 (13-16 μm fibre cell diameter). Moreover, some of the RILs with high fibre content had fibre cell diameters comparable to that of fibre flax cultivars. Comparable fibre cell diameters between the two flax types were found in a study by Lamb and Denning (2004), indicating ambiguity regarding the actual sizes of fibre cells in the two flax types.

Some of the traits that were significantly higher ($p < 0.01$) in the fibre flax group relative to the low fibre group were average area of single fibre cells, total fibre area and stem area at all growth stages and in both the Kernen Farm and growth chamber studies (Table 4.3). Similar differences were also observed between Viking and E1747 at two out of the three growth stages (Tables 4.4.1- 4.4.3), indicating the importance of these traits in differentiating fibre flax from oilseed flax. Though the differences still existed between the fibre flax group and high fibre group, those differences were in lower magnitude due to improved fibre content of the stems as evidenced by their NIR fibre content (Tables 4.1 and 4.3).

High fibre RILs vs. Low fibre RILs

The contrast analysis revealed greater differences between high fibre and low fibre RIL groups in the growth chamber study as compared to the Kernen Farm, which might be due to the presence of greater variability under field conditions such as temperature and soil moisture, confirming the polygenic nature of the fibre related traits and the importance of environment during fibre deposition. Dimmock et al. (2005) also supported the importance of environment in influencing fibre deposition in plants. Except in one or two occasions, average area of single fibre cell, total fibre area, phloem area and fibre to stem area ratio were significantly greater in the high fibre RILs at all growth stages and in both Kernen Farm and growth chamber studies (Figure 4.4, Tables 4.4.1 – 4.4.3). Furthermore, the differences became more prominent with increased level of significance from green capsule stage onwards, indicating the importance of this growth stage for selecting high fibre genotypes in controlled environments such as a growth chamber. This result was also confirmed in correlation analysis conducted at the green capsule stage, which indicated a strong relationship between the Kernen Farm and growth chamber studies for average area of single fibre cells ($p < 0.0001$), total fibre area ($p < 0.01$) and fibre to stem area ratio ($p < 0.05$) (Table 4.6). Previous studies have also indicated the importance of the green capsule stage during harvesting for linen production in fibre flax (Barber, 1991, Deyholos, 2006).

Table 4.6. Pearson correlation coefficients of specific anatomical traits between the Kernen Farm and growth chamber study conducted at the green capsule stage.

	Green Capsule Stage **Growth chamber vs. Kernen Farm**	**Correlation coefficient (r)**	**p-value**
1.	Average area of single fibre cell	0.65	< 0.0001
2.	Total fibre area	0.47	< 0.01
3.	Fibre to stem area ratio	0.38	< 0.05
4.	Number of fibre cells	0.13	n.s

Dual purpose RILs

Previous studies have shown the possibility of identifying and developing dual purpose varieties, which could be used both for bast fibre as well as seed oil extraction (Foster et al., 1997). Though in the growth chamber study, the dual purpose RILs had significantly lower fibre to stem area ratio and fibre to xylem area ratio from green capsule stage onwards, these differences were not observed in the Kernen Farm study (Table 4.4.2 and Table 4.4.3). The dual purpose RILs fell under the intermediate category between the high fibre and low fibre RILs as they did not show any consistent significant difference from both the groups, throughout the study. This outcomes is also supported by the NIR fibre % results (Table 4.1, Figure 4.4), which showed intermediate fibre contents between high fibre and low fibre groups for the dual purpose lines. Interestingly, cortex width was the only anatomical trait that was significantly higher in the dual purpose group relative to the high / low fibre group from green capsule stage onwards in both the Kernen Farm and growth chamber studies. This region of the stem may act as an anatomical marker for selecting dual purpose RILs under diverse environments (Table 4.4.1). However, further studies would be required to support this hypothesis.

Short RIL vs. Tall RIL

The differences in the stem anatomy of short vs. tall RIL genotype were not consistent over stages or environments but tended to differ significantly only by the amount of xylem area or traits related with xylem area such as fibre to xylem area ratio and xylem to stem area ratio (Tables 4.4.1 – 4.4.3). This result is supported by the fact that, in most woody plants xylem provides the mechanical support, which enables the plant to grow tall (Ye, 2002). In addition, the amount of lignin, which is an integral part of secondary xylem has been shown to decrease plant height in sorghum and lodging in peas (Pederson et al., 2005; Banniza et al., 2005). A recent study using plant growth regulator treatments also indicated a significant association between plant height and length of the xylem fibres in the flax stems. There was, however, a lack of significant association between plant height and bast fibres when treated with plant hormones such as gibberellic acids and auxins in the flax stems (McKenzie and Deyholos, 2011).

Apart from having differences in xylem, the short type RIL exceeded the tall type RIL in fibre content as estimated by NIR (Table 4.1). The mean values of the short RIL had

significantly greater total fibre area and fibre to stem area ratio as compared to the tall genotype at two out of three growth stages and in either the Kernen Farm or growth chamber studies. This result indicated the importance of taking fibre concentration or fibre to stem area ratio as opposed to plant height; and total stem/dry matter yield for assigning criteria for high fibre genotypes selection. A study conducted by Sankari (2000a) also showed that taller plants with good dry matter production did not necessarily produce higher fibre yields and that other agronomical factors may also influence total fibre yields.

Correlation analysis

Correlation among anatomical traits

A correlation analysis done at the seedling stage indicated some disparities between the direction and magnitude of correlations between the Kernen Farm and growth chamber studies. However, at later stages of plant growth these disparities disappeared and overall, most of the traits followed the same pattern in direction and magnitude of the relationship. Strong positive correlations were found between fibre to stem area ratio, total fibre area, average area of single fibre cell and number of fibre cells (Table 4.5.1 – 4.5.3). Further regression analysis for the above mentioned traits ($R^2 > 50$ %, $p < 0.01$) confirmed the extent of strong association among these anatomical traits (Figures 4.5 and 4.6). Very few negative correlations were found but were not significant in one or both Kernen Farm and growth chamber studies, indicating the absence of any tight undesirable linkage between the anatomical traits.

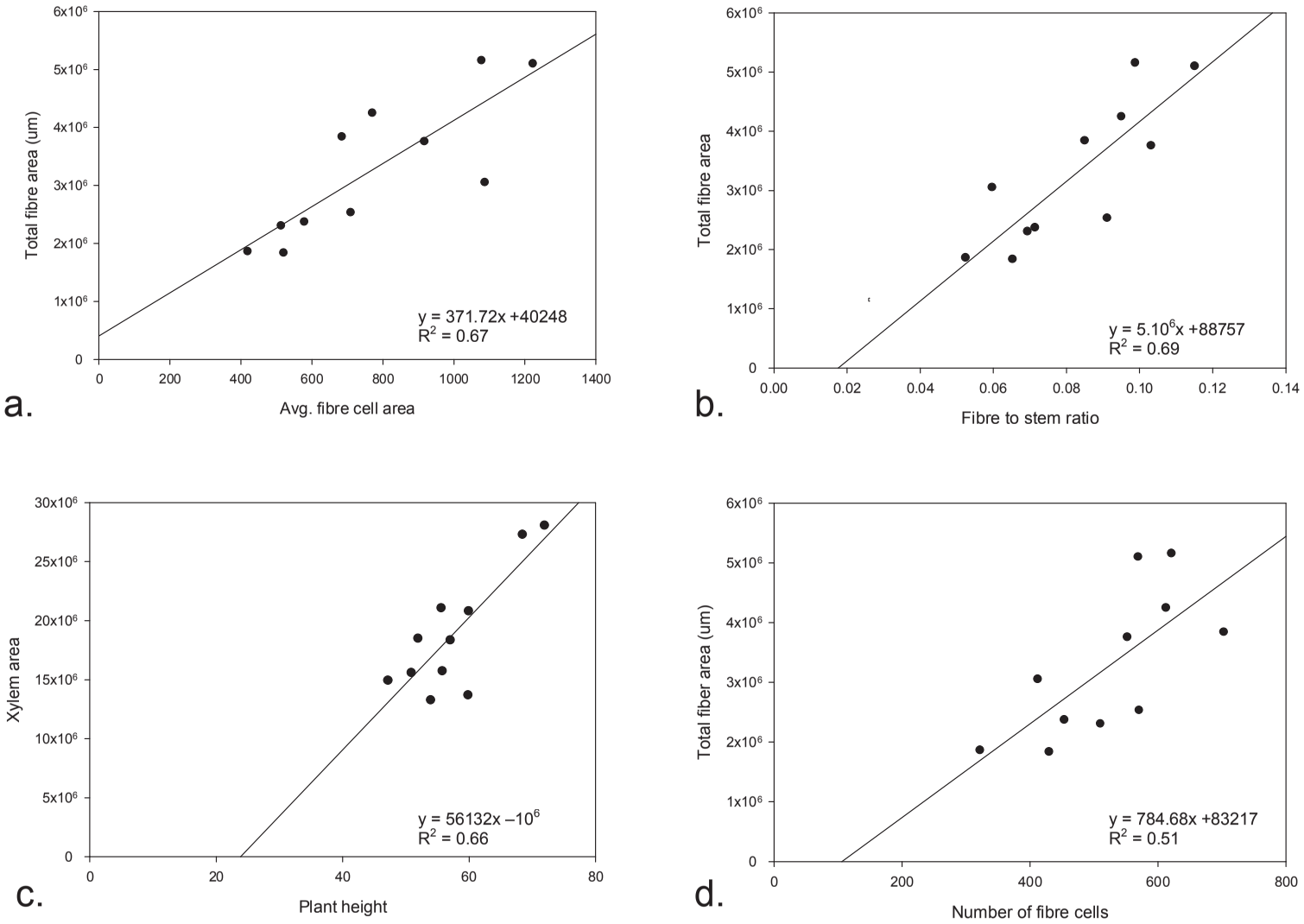


Figure 4. 5. Regression coefficients (R^2) and regression equation (y) between different anatomical traits (averaged over Kernen Farm and growth chamber) at physiological maturity stage.

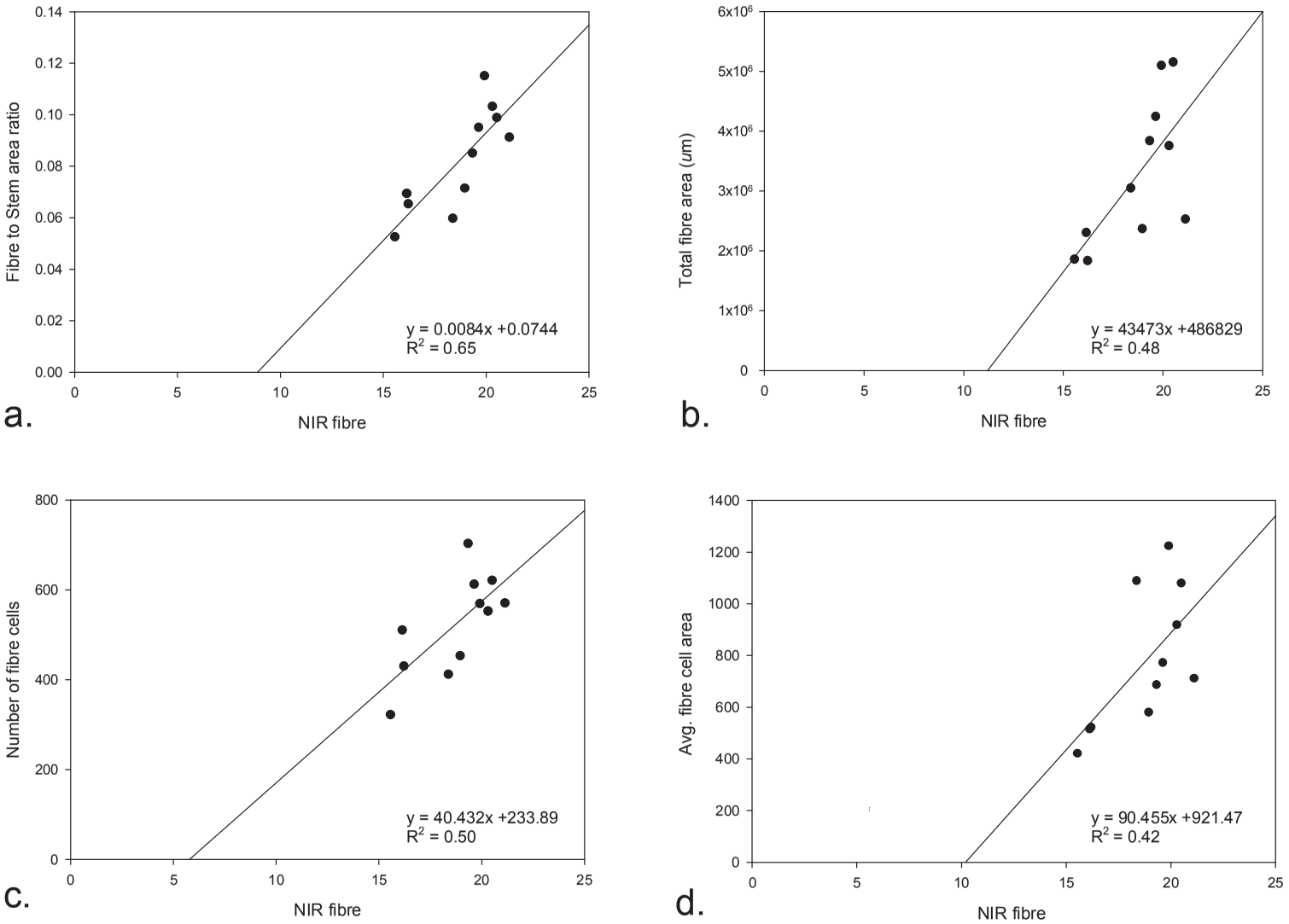


Figure 4. 6. Regression coefficients (R^2) and regression equation (y) between NIR fibre content and different anatomical traits (averaged over Kernen Farm and growth chamber) at physiological maturity stage.

Correlation analysis between anatomical traits and the multi-location field traits (Chapter 3)

Plant height from the 2006 multi-location field trial correlated with only two anatomical traits, xylem area ($p < 0.01$) and stem area ($p < 0.01$), at the physiological maturity stage in both the Kernen Farm and growth chamber studies (Table 4.5.2 and Table 4.5.3). This finding was also supported by the contrast analysis (Tables 4.4.1 - 4.4.3, Figure 4.5) and regression analysis between xylem area and plant height as discussed earlier.

Fibre content as estimated by NIR from the 2006 multi-location trial was highly correlated ($p < 0.0001$) with fibre to stem ratio, total fibre area in the stem, average area of single fibre cell and number of fibre cells in both the Kernen Farm and growth chamber studies over all growth stages but more prominently at the physiological maturity stage (Tables 4.5.1 – 4.5.3, Figure 4.6). Similar correlations were also observed by Shepherd (1956), who concluded that fibre concentration was significantly correlated with fibre bundle area and ultimate fibre numbers in the bundle. The correlation analysis presented here, confirmed the assumptions anatomically that the NIR instrument was actually measuring only the fibre region in the stem and not other anatomical regions such as epidermis, cortex, cambium, xylem or pith region (Table 4.5.3).

However, it must be noted that the magnitude of these relationships was lower, indicative of the polygenic nature of the fibre trait, which has also been seen in previous research (Keijzer and Metz 1992, Easson, 1989; Scheer-Treibel et al., 2000). Also the fibre estimation by NIR and plant height measurements were based on multi-location field trials consisting of hundreds of plants while the anatomical measurements were taken from a very small set of plants and from single cross section of the stems. Nonetheless, strong regression coefficients ($R^2 > 40$ %) between the above mentioned anatomical traits and NIR fibre content, confirmed the importance of these anatomical traits / markers in fibre concentration estimation (Figure 4.6).

This study established an anatomical basis for further research into flax stem anatomy and fibre content improvement. The results indicated that even though there were significant differences between fibre and oilseed flax lines, there was a possibility to reduce this gap by selecting high fibre oilseed flax lines based on anatomical markers such as the average area of single fibre cells, number of fibre cells, total fibre area and fibre to stem area ratio. Consequently, this study also supported the use of controlled environments such as growth

chambers for the purpose of growing and quick screening of genotypes at the green capsule stage.

Correlation studies have confirmed that anatomical methods are as useful as NIR estimations for identifying high fibre genotypes. Also a lack of significant correlation between plant height and any of the fibre related anatomical regions suggest that breeding high fibre genotypes in short Canadian oilseed flax varieties is possible.

CHAPTER 5

Preliminary molecular marker analyses of flax (*Linum usitatissimum* L.)

Abstract

The study aimed to characterize a 95 entry recombinant inbred line (RIL) population derived from a cross between a fibre flax (Viking) and an oilseed flax genotype (E1747- an EMS mutant) using molecular markers. Nineteen molecular markers (17 simple sequence repeats and 2 cleaved amplified polymorphic sequences) were used to assess the extent of genetic variability in the RIL population. Twelve markers were distributed over five linkage groups using MAPMAKER software. The remaining seven markers were found to be unlinked. The highest association ($p < 0.0001$) between phenotypic data and molecular data was found for linoleic and linolenic acid concentration with molecular markers LuFAD3A and LuFAD3B using both single marker analysis and stepwise regression analysis. Molecular marker analyses confirmed the potential of Viking × E1747 population for identifying genes / markers related to both fibre as well as oilseed traits.

Introduction

Flax (*Linum usitatissimum*), is an annual self-pollinating crop cultivated for its seed oil (oilseed) and for its fibre (fibre flax). New applications of flax fibre such as composites, pulp and paper making do not require the very long or fine fibres desired by the textile industry (Foster et al., 1997). This raises the possibility of using the same plant for producing both fibre and seed oil. Exploitation and characterization of such dual purpose varieties will require both phenotypic as well as genotypic evaluation.

Flax oil production and fatty acid profiles have been studied for a long time using conventional breeding approaches. Flax seed oil content is a quantitative trait significantly influenced by environmental interaction (Comstock, 1966; Comstock et al., 1969). Two independently inherited genes control the low-linolenic acid trait in flaxseed which makes it suitable for edible uses (Rowland, 1991). Genetic studies have also been done on the seed color inheritance (Mittapalli and Rowland, 2003) and flower color (Sood et al., 2007) in flax. Unfortunately, information about the genetic architecture of stem fibre concentration is not clear and is

suspected to be greatly influenced by the environment (Popescu et al., 1998) making it unsuitable for phenotypic selection. This limitation can largely be overcome by the use of molecular markers. Molecular markers are powerful tools for gaining insight into the inheritance of quantitatively inherited characters. They are also useful for variety identification and evaluation of DNA variation. As a result, molecular markers are used for both the 'dissection' of complex agronomic traits and for the development of marker-assisted breeding strategies.

A series of different molecular marker systems, which became available during the last two decades, can be broadly placed under three classes: (i) the first generation molecular markers, including random amplified polymorphic DNA (RAPDs), restriction fragment length polymorphism (RFLPs); (ii) the second generation molecular markers, including simple sequence repeat (SSRs), amplified fragment length polymorphism (AFLPs) and (iii) the third generation molecular markers including expressed sequence tags (ESTs) and single nucleotide polymorphism (SNPs) (Gupta et al., 2001). In previous research studies with molecular markers, RAPD (Fu et al. 2002, 2003a, 2003b), RFLP (Oh et al. 2000), AFLP (Spielmeyer et al. 1998; van Treuren et al., 2001; Everaert et al., 2001; Adugna et al., 2005 , 2006; Vromans, 2006) , inter simple sequence repeats (ISSR) (Wiesner and Wiesnerová, 2004), and microsatelite markers (Roose-Amsaleg et al., 2006) have been developed to analyze flax genomics. However, their numbers are limited as compared to other major crops.

Despite these advances, RAPD markers have low repeatability, whereas RFLP and AFLP markers are quite labor intensive. SSRs, on the other hand are easy to use, evenly distributed, highly reproducible and co-dominant (Powell et al. 1996a). SSRs, also called microsatelites, consist of a variable number of tandem repeats. SSR markers can be derived from either genomic sequences or ESTs. EST-based SSRs (EST-SSRs), also called genic SSRs, are particularly informative because they tag the expressed gene from which they were derived and can be used as diagnostic markers for the genes or for molecular mapping.

EST-SSRs have been successfully used in cotton (Han et al., 2004; Park et al., 2005; Han et al., 2006), jute (Mir et al., 2008), soyabean (Hisano et al., 2007), barley (Varshney et al., 2006), rice (La Rota et al., 2005), and wheat (Yu et al., 2004; Peng and Lapitan 2005; Fu et al., 2006). EST resources in flax are relatively new and limited. About 900 ESTs from the fibre-bearing stem tissues of fibre flax cultivar Hermes have been submitted to GenBank by Day et al.

(2005b). Subsequently, Roach and Deyholos (2007) produced a 9,600 cDNA clone library from the stem peels of Norlin flax and submitted 1,462 ESTs into GenBank. In two independent variety identification studies, a total of 37 flax SSR markers were developed by Wiesner et al. (2001) and Roose-Amsaleg et al. (2006). A study by Vrinten et al. (2005) also identified two cleaved amplified polymorphic sequence (CAPS) markers linked to the low linolenic acid trait in flax seed.

In less well studied crops such as flax, a paucity of markers is a hindrance in constructing genetic maps. It is, however, important to use available information to identify important molecular markers for their possible use in marker-assisted selection (MAS). For this purpose, a recombinant inbred line (RIL) population consisting of 95 lines was developed by crossing a fibre flax cultivar (Viking) with an oilseed mutant line (E1747). The above mentioned on-line database of publicly available flax ESTs (Day et al., 2005b; Roose-Amsaleg et al., 2006; Roach and Deyholos, 2007) as well as the CAPS markers (Vrinten et al., 2005) were tested in the RIL population segregating for both fibre and oil related traits using a single-marker analysis approach (Chandra et al., 2004). The objectives of the present research were (1) to identify polymorphic SSR markers in the Viking × E1747 RIL population; (2) to identify potential QTL-harboring markers for use in MAS and (3) to study the population structure of the flax RIL population using molecular markers.

Materials and methods

Plant materials, phenotyping and genotyping

A set of 95 RILs developed by Dr. Steven Knapp, University of Georgia by crossing a fibre flax cultivar (Viking) with an oilseed mutant line (E1747) were evaluated in this study. The RIL population was developed using the single seed descent method from a F_2 derived F_6 generation. Viking is a European fibre flax cultivar, expected to have high fibre concentration and plant height but inferior oil characteristics as compared to the Canadian oilseed varieties. E1747 is an ethylmethane sulphonate (EMS) mutant line from McGregor oilseed flax with high oil concentration and < 5 % linolenic acid content making it suitable for commercial edible-oil markets.

Field trials were conducted at Kernen Crop Research Farm in 2005 and over three Saskatchewan locations (Saskatoon, Floral, and Melfort) in 2006. A randomized complete block design was used with two replications each in the 2006 multi-location trial. Traits under study included stem fibre concentration, plant height at maturity, total seed oil concentration, total protein concentration and seed fatty acid composition. Fatty acids measured in the study were palmitic, stearic, oleic, linoleic and linolenic.

Molecular marker procedures

DNA extraction procedure

For each of the 95 RILs, seedlings were germinated in a Petri-dish from 15-20 seeds. After five days of germination, 50-100 mg of seedling tissue was obtained by pooling the 20 individuals. Tissues were collected in 2 mL Eppendorf tubes and immediately frozen in liquid nitrogen. All samples were stored at -80 $^{\circ}$C for further use.

Samples were freeze- dried overnight after which five glass pearls were added to each Eppendorf tube and tissues ground mechanically into a fine powder. Subsequently, total genomic DNA was isolated using a CTAB method (Doyle and Doyle, 1990). Extracted DNA was re-suspended in 50 -100 μL of TE buffer (10 mM Tris pH 8.0 / 1 mM, EDTA (pH 8.0)), depending on the size of the pellet, and stored at 4 $^{\circ}$C. One microlitre of each sample was loaded on a 1 % agarose gel to estimate DNA quality. Spectrophotometer determination was also used to assess the quality and the quantity of the sample DNA.

Detection of SSRs

927 ESTs produced from stem fibre tissues (Day et al., 2005b) were used in this study to detect SSRs. These ESTs were derived from the NCBI GenBank (CV478070 - CV478996) and mined for the presence of putative SSRs using a PERL based computer program (http://www.gramene.org/db/markers/ssrtool; November 5th, 2006). The program detected sequence repeats based on the following criteria: dinucleotide repeat ≥ 5; trinucleotide repeat ≥ 4; tetranucleotide ≥ 3; pentanucleotide ≥ 3. Primer pairs were automatically designed with parameters as described by primer3 (http://www.ncbi.nlm.nih.gov/tools/primer-blast/, November 5th, 2006) with 18 - 23 nucleotides primer length, devoid of secondary structure or

consecutive tracts of a single nucleotide, a GC content of 50 % (T_m approximately 50 – 70 ˚C), maximum T_m difference as 20 ˚C, maximum 5' complementarity - 8 and maximum 3' complementarity - 3.

Thirty eight additonal SSR markers developed by Roach and Deyholos (2007) from 1,462 EST sequences submitted to NCBI GenBank (EH791145-EH792584 and EL585326-EL585347) along with 23 SSRs from Roose-Amsaleg et al. (2006), 9 ISSRs developed by Wiesner and Wiesnerová (2004), and 2 CAPS markers developed by Vrinten et al. (2005) were also screened for polymorphism among the parents and the RIL population. A list of all the markers screened in this study is listed in Appendix Tables C.1- C.4.

PCR amplification

Non-redundant primer pairs were used in PCR experiments to confirm amplification potential. All primer pairs were originally tested at an annealing temperature of either 50˚C or 55˚C, and those that did not amplify well were then evaluated at 61˚C or 67˚C. PCR amplifications were performed in a Applied Biosystems thermocyler, with 37 cycles at 94˚C for 1 min, 55-60˚ C for 1 min and 72˚C for 10 min. Each reaction (25 µl) contained 2.5 µl of 10x PCR buffer, 200 µM of each dNTP, 0.2 µM of each primer, 1 U of *Taq* DNA polymerase and approximately 100 ng of sample DNA. PCR products were then analysed in 6 % polyacrylamide gels and visualized by autoradiography.

Data analysis

Single marker analysis (SMA) by linear regression and Fisher's method for analysis of variance was carried out to establish the association of markers with phenotypic traits using the SAS statistical program (Statistical Analysis Software, SAS institute, Cary, NC). Regression coefficients (R^2) values were also determined to assess the variability by considering the phenotypic data as dependant and molecular marker data as independent variables. SMA was conducted on the 2005 Kernen data and on multi-location field data in 2006 pooled over the three locations. Stepwise regression analysis (SWR) was performed on the multi-location 2006 phenotypic data and the marker data to confirm the association between the markers and the phenotypic traits. SWR method usually selects fewer markers than SMA, with less false positives, but tends to pick up markers that may be linked (Chandra et al., 2004; Murthy et al.,

2005). The molecular marker alleles were converted into a binary matrix for regression analysis where the marker score was 0 or 1 based on the marker genotypes of E1747 and Viking.

Linkage mapping

MAPMAKER/Exp software 3.0 (Lincoln et al., 1993) was used to perform genetic linkage mapping between polymorphic SSR markers. For linkage analysis, the molecular marker alleles were converted into a matrix containing A, B and H notation corresponding to Viking, E1747 and heterozygote marker genotype, respectively.

Phylogenetic tree

All polymorphic marker bands were scored for the presence or absence of unique and shared polymorphic products. This information was then used to generate a similarity coefficient. The molecular marker alleles were converted into a binary matrix where the marker score was 0 or 1 based on the marker genotype of E1747 and Viking, respectively. The score for any missing data was given the value -999 as per NTSYSpc version 2.2 (numerical taxonomy and multivariate system program) data format (Exeter Software, Setauket, NY). The NTSYSpc version 2.2 was used to estimate genetic similarities with the Dice coefficient (Dice, 1945). The matrix of similarities were calculated among all possible pairs of RILs using unweighted pair-group method arithmetic average (UPGMA) method. A dendogram was generated with the sequential agglomerative hierarchical and nested (SAHN) method to identify similarity/ dissimilarity between the 95 RILs and their parents.

Results

Field evaluation

Analysis of the data revealed a wide range of variation among the traits of the 95 RIL population in both years and locations. Histograms based on the frequency distribution of the pooled mean values (Figure 5.1.1 and Figure 5.1.2) revealed that fibre concentration, plant height, total oil concentration, total protein concentration, palmitic acid concentration and oleic acid concentration followed a bell shaped continuous single peak symmetrical distributions while stearic acid concentration, linoleic acid concentration and linolenic acid concentration showed

double peaked frequency distributions. A list of mean phenotypic data from the 2006 multi-location field trial is attached in Appendix A.

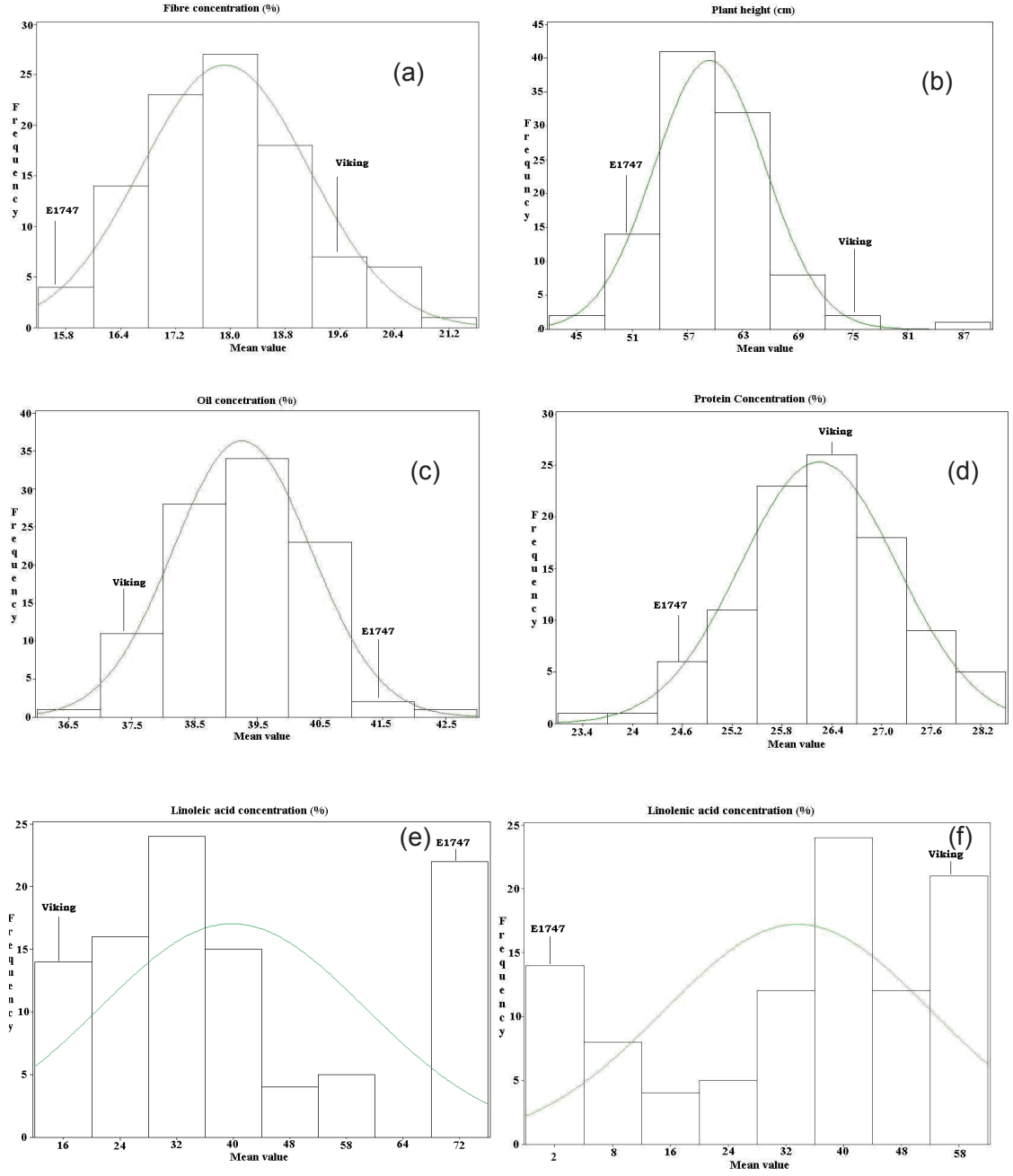


Figure 5.1.1. Histograms and frequency distribution of the RIL population for (a) fibre concentration, (b) plant height, (c) oil concentration, (d) protein concentration, (e) linoleic acid concentration and (f) linolenic acid concentration grown in 2006 multi-location trial.

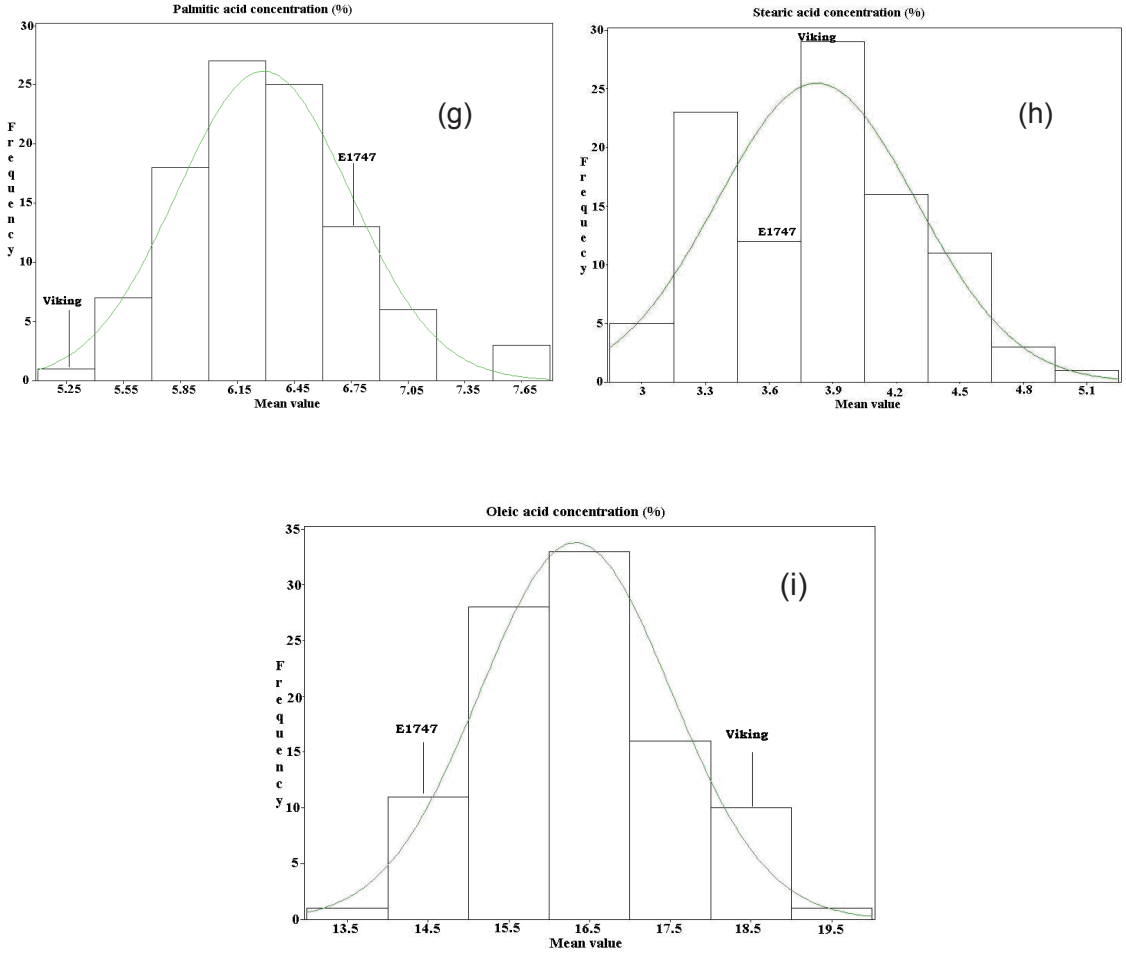


Fig 5.1.2. Histograms and frequency distribution of the RIL population for (g) palmitic acid concentration, (h) stearic acid concentration and (i) oleic acid concentration, grown in 2006 multi-location trial.

Molecular marker evaluation

This experiment was designed to identify molecular markers for loci controlling both stem fibre as well as seed oil related traits in flax. The source of high stem fibre and plant height character was the parent "Viking", whereas the source of good seed oil quantity and quality was the parent,"E1747".

A total of 927 ESTs were screened for the presence of EST-SSRs from the Day et al., (2005b) EST collection of flax stem fibre tissues. Out of these, 241 were found to contain SSR repeats. However, only 143 primers (60 %) amplified regions with an intense and reproducible PCR banding pattern. The EST-SSRs had in general more trinucleotide repeats as compare to di, tetra, or penta nucleotide repeats ranging from 111 bp to 384 bp in length.A list of all the EST-SSRs is attached in Appendix Table C.1. Only one EST-SSR, CV8824, was found to be polymorphic between the parents and the population.

A total of 38 SSR markers developed from stem fibre-enriched bast tissues (Roach and Deyholos, 2007) were evaluated for the presence of polymorphism between the parents (Appendix C, Table C.2). Nine markers out of the 38 failed to produce intense and reproducible bands. Out of the remaining 29 markers, eight were found to be polymorphic (28 %) while the remaining (21) were monomorphic (Figures 5.2.1 and 5.2.2). The annealing temperatures of these markers ranged from 53^{o}C - 57^{o}C. Another set of 23 SSRs reported by Roose-Amsaleg et al. (2006) produced from the developing ovules of flax seed were also tested for presence of polymorphism across the RIL population (Appendix C,Table C.3). Seven markers out of the 23 (30.4 %) were polymorphic.. The annealing temperature for the seven markers was 55^{o}C. Nine ISSR markers developed by Wiesner and Wiesnerová (2004) (Appendix C, Table C.4), were evaluated in this population, however, all were monomorphic.

Two CAPS markers linked with the low linolenic acid trait developed by Vrinten et al. (2005) were also tested on the parents and the progeny to find specific RILs having good edible oil characteristics. Screening of the population revealed the presence of a heterozygous type banding pattern in 13 out of 95 (13.7 %) in case of the LuFAD3A marker and 8 out of 95 RILs (8.4 %) in case of the LuFAD3B marker (Figure 5.3). A list of all the polymorphic markers found in the RIL population can be found in Table 5.1.

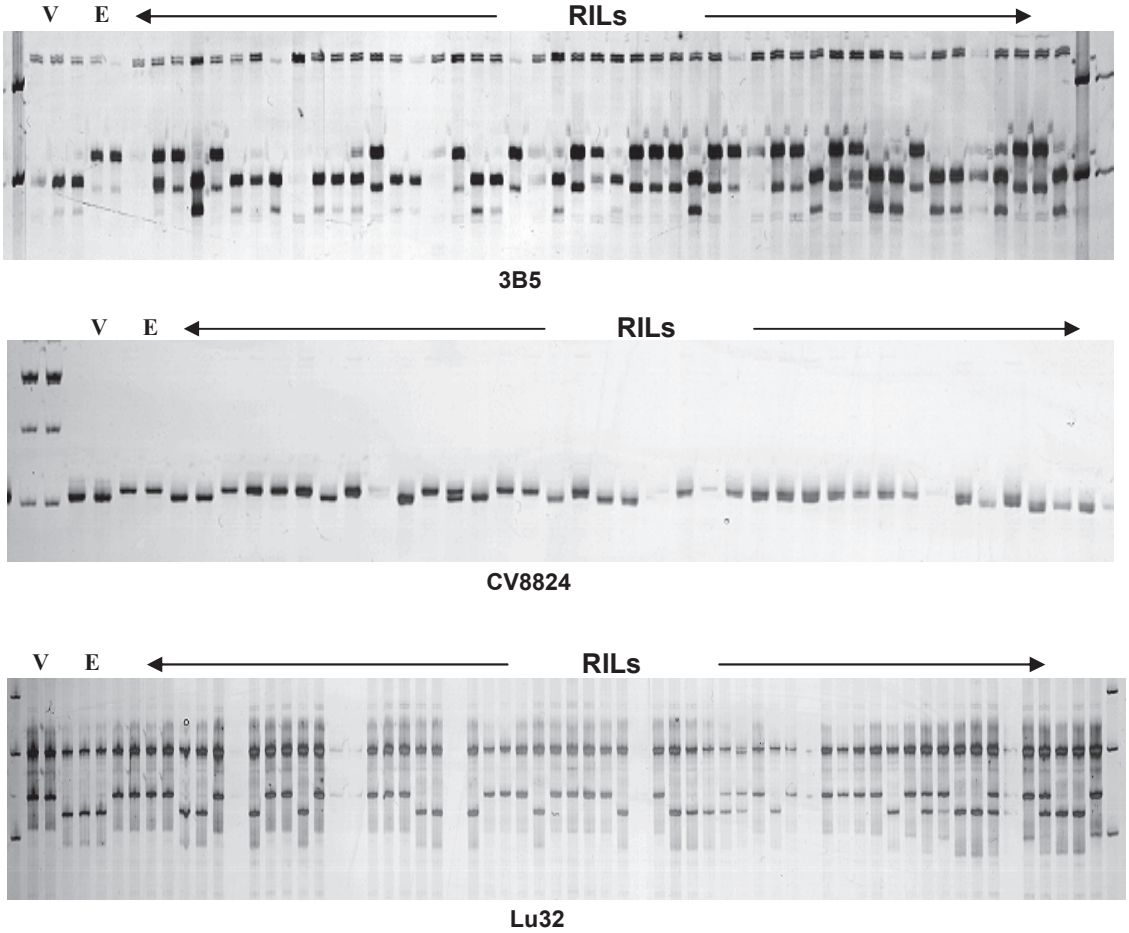


Figure 5.2.1. Marker segregation of three different SSR markers in a subset of the RIL population and the parents (V=Viking, E= E1747) detected on a 6% PAGE gel.

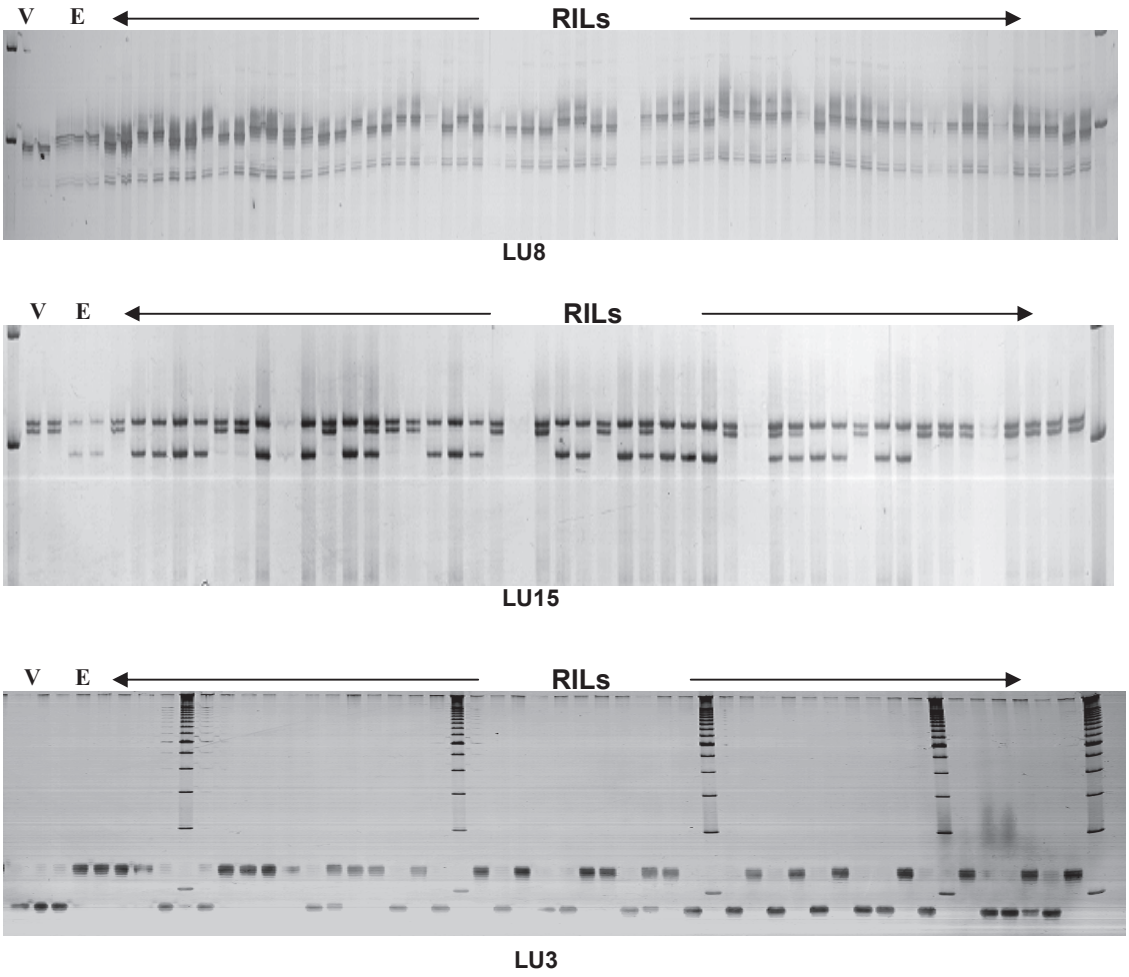


Figure 5.2.2. Marker segregation of three different SSR markers in a subset of the RIL population and the parents (V=Viking, E= E1747) detected on a 6% PAGE gel.

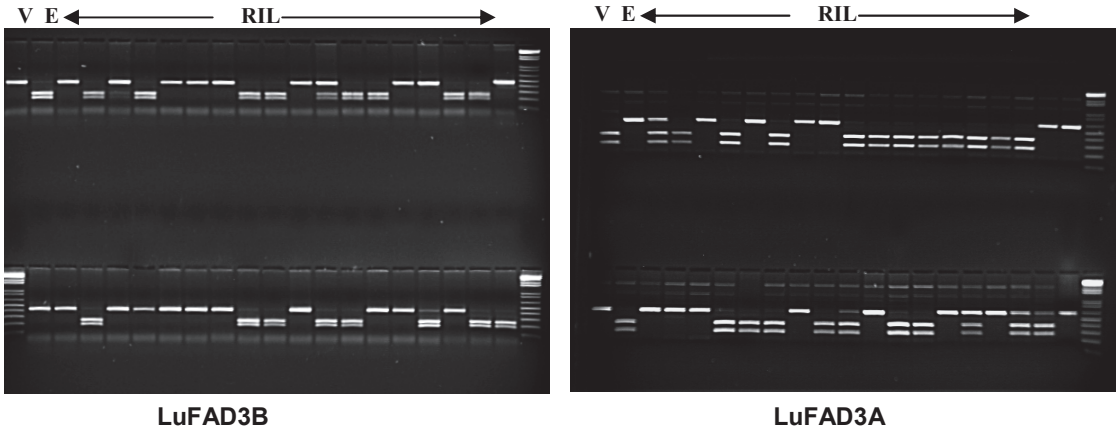


Figure 5.3. Marker segregation of LuFAD3A and LuFAD3B CAPS markers (Vrinten et al., 2005) in a subset of the RIL population and the parents (V=Viking, E= E1747) detected on a 1.5 % agarose gel.

Table 5.1. List of EST- SSR and CAPs markers along with their primer information found polymorphic among the parents and the RIL population identified in the molecular marker study.

Sl.No	Marker	Ann temp	Type	Rep len	Size(bp)	Forward primer	Reverse primer
1	LuFAD3A	61°C	CAPS	-	1475	CAGTGACCTGTTCGCACCG	CCCGGCTAGGGTGATCATG
2	LuFAD3B	61°C	CAPS	-	1328	TTCAAAACTGGGCTCTGCAG	TCACATTGTTCAACAACCAGA
3	CV8824	58°C	SSR	(CTAG)5	254	CATTGGTTACACCCTTGTCT	GGTTGATGGTATTGGGTAGA
4	LU3	55°C	SSR	(GT)11	158	CTTTTTTGAGTCACCAAGCC	CGCTGGAGTCTGAATCCTAG
5	LU8	55°C	SSR	(AG)24	216	ACACTTGCTATTAGCTACAAGAGAG	CAGCATCCAGAGGTTCTCAC
6	LU15	55°C	SSR	(CAT)8	208	GGGTTATACATTGTTCTTCATTCGG	CAAGAGGAATGCAGGATGCC
7	LU28	55°C	SSR	(TCT)8	187	TCCCAGCGAGTTTGGTGAG	TGGAGGAACTAATTGTGGCAAG
8	LU31	55°C	SSR	(TC)8	138	CACGCAATCTCTCCCAGACA	GAGAGTTTAGGGTATGCACTGA
9	LU32	55°C	SSR	(AG)10	252	GAAAAGGAAGGCTTAGAAGAAGAAG	AGTTTCTCAATACACAGATCGAAGG
10	LU38	55°C	SSR	(GAA)8	168	TTACAATACGAAGACTTCGATCCC	TTTGCTTCGGTTAATGGCC
11	5B6	55°C	SSR	(GTCT)9	152	TGAGTGGGTTAGTGGGATCTG	AAGCAGCACGAACCTGTTTT
12	3B5	57°C	SSR	(GACA)8	174	CGAGATATATTGGGATGGATCA	TACTGGGGTTTCGTTTCCTG
13	6H5	57°C	SSR	((TC)22	152	TATCCTTGGCATTACGGTCTG	GAAGGCTGTAATTAAGGGTGGT
14	8F9	57°C	SSR	(TC)20	115	AACTCCCCTTCATCCTTTCT	TGATAGTATGATTTGGTTGGAAGG
15	2D2U	55°C	SSR	(CT)17	104	CATCCAACAACTTTTCGAGGA	GCCTCAGATAATCGAAGAATCA
16	2D2L	55°C	SSR	(CT)17	104	CATCCAACAACTTTTCGAGGA	GCCTCAGATAATCGAAGAATCA
17	7F2	57°C	SSR	(GACA)8	111	TTGGGATGGATCAATTCAGTT	CCTTTCATGCAAATGCTTTC
18	8A11	53°C	SSR	(CAGA)9	117	ATGAAGCAGCACGAACCTGT	GAGTTGGTTAGTGGGATCTGT
19	9B2	60°C	SSR	(GT)11	103	TCTTAAATGGGGGTTTTCGA	CAAACACTTGCTGGTTAGTTGG

Single marker analysis

Linear regression analysis was used to explain the phenotypic variability of the population with respect to the 19 molecular markers. The coefficient of determination (R^2) as well as probability levels based on F-tests were calculated for the 19 markers and 9 phenotypic traits as previously described. The distributions of fibre concentration, plant height, oil concentration, protein concentration were normal and showed transgressive segregation. However, the distributions of linolenic acid and linoleic acid concentration were discrete and showed only a few trangressive segregants (Figure 5.1.2). The regression coefficent (R^2) discussed in this text refers to the SMA analysis calculated using 2006 multi-location phenotypic data. Regression coeffients calculated using both SMA and SWR methods are listed in Table 5.2.

Only one marker namely, LU8 (R^2= 0.055) was associated with fibre concentration at $p < 0.05$ using the 2006 multi-location field/ phenotypic data following both SMA and SWR methods (Table 5.2). However, this marker did not show association with fibre concentration at the 2005 Kernen Farm and 2006 Melfort field trials when analysed separately. LuFAD3B (R^2= 0.117) and CV8824 (R^2= 0.095) were found closely associated with plant height ($p< 0.01$). These two markers were also found significantly associated with plant height when compared to single locations as well as using the SWR method.

The highest association for seed oil related traits were found in LuFAD3A and LuFAD3B CAPS markers for linolenic acid and linoleic acid concentration ($p < 0.0001$) in all three regression analyses (Table 5.2). LuFAD3A was significantly associated with palmitic acid concentration (R^2= 0.199, $p <0.0001$) and oleic acid concentration (R^2_{SMA}= 0.185, $p < 0.0001$) in all three analyses. LuFAD3B was significantly associated with palmitic acid concentration (R^2= 0.079, $p <0.01$) and oleic acid % (R^2= 0.074, $p < 0.01$) in all three regression analyses. LU32 was also significantly associated with palmitic acid concentration (R^2= 0.107, $p < 0.01$) for all three regression analyses. LU32 was the only marker that was found to be significantly associated with protein concentration (R^2= 0.083, $p < 0.01$) in all three regression analyses. None of the markers, however, showed an association with stearic acid concentration using either SMA or SWR analysis.

Five different SSR markers had significant associations with oil concentration. The highest association was found with 5B6 ($R^2 = 0.104$) followed by LU3 ($R^2 = 0.072$) and 8A11 ($R^2 = 0.068$) at $p < 0.01$ as per the SMA analysis using 2006 multi-location pooled data. In addition, two other markers 2D2U ($R^2 = 0.065$) and LU31 ($R^2 = 0.058$) also showed some association with oil concentration at $p < 0.05$. However, the SWR analysis revealed that the most desirable marker combination with respect to oil concentration was 2D2U and 5B6 (Table 5.2).

Linkage map

A total of 12 markers were distributed over five linkage groups using MAPMAKER software. The remaining seven markers were unlinked. The linkage group with the greatest number of markers linked to it was linkage group 4 with a relative distance of 3.2 cM between 3B5 and 7F2 ; 7.2 cM between Lu28 and 3B5 and 15.8 cM between Lu31 and Lu28. All other relative distances between the linked markers are listed in Table 5.3. Linkage group 5 consisted of two linked markers 5B6 and 8A11 with a relative genetic distance of 4.9 cM. It is interesting to note from the previous SMA analysis that these two markers were also highly associated ($p < 0.01$) with oil concentration trait (Table 5.2).

Table 5.2. Marker association with different traits in 2005 and 2006 field experiments and their regression value using different model selection criteria.

Markers			Fibre %	Plant height	Oil %	Protein %	Palmitic %	Oleic acid %	Linoleic acid %	Linolenic acid %
	SMA	2006		0.117**(M,F,K)			0.079**(M,F,K)	0.074**(M,F,K)	0.508***(M,F,K)	0.510***(M,F,K)
LuFAD3B		2005		0.58*			0.129**	0.012ns	0.481***	0.486***
	SWR	2006		**			**	**	***	***
	SMA	2006					0.199***(M,F,K)	0.185***(M,F,K)	0.341***(M,F,K)	0.332***(M,F,K)
LuFAD3A		2005					0.178***	0.062*	0.359***	0.354***
	SWR	2006					***	***	***	***
	SMA	2006			0.065*(M,F)					
2D2U		2005			0.044ns					
	SWR	2006			**					
	SMA	2006		0.095**(M,F,K)						
CV8824		2005		0.083**						
	SWR	2006		**						
LU3	SMA	2006			0.072**(M,F,K)					
		2005			0.071*					
	SWR	2006			*					
	SMA	2006	0.055*(F,K)							
LU8		2005	0.025ns							
	SWR	2006	*							
	SMA	2006			0.058*(M,F)					
LU31		2005			0.081*					
	SWR	2006			*					
	SMA	2006				0.083**(M,F,K)	0.107**(M,F,K)			
LU32		2005				0.082*	0.071**			
	SWR	2006				**	**			
	SMA	2006			0.104**(M,F,K)					
5B6		2005			0.144**					
	SWR	2006			**					
	SMA	2006			0.068**(M,F,K)					
8A11		2005			0.130**					
	SWR	2006			ns					

Single marker analysis (SMA) using simple linear regression; SMA-2006 refers to the regression analysis performed using the pooled mean phenotypic values from 3 location viz., Melfort (M), Floral (F) and Kernen (K); SMA-2005 refers to the regression analysis performed using the mean phenotypic values from 2005 year Kernen location data ; SWR= Stepwise regression based on pooled 2006 year data; the alphabets M,F,K refer to the respective locations in which the analysis were found significant when treated individually.

*p<0.05, **p<0.01, ***p<0.0001, ns= non significant

Table 5.3. Linkage groups and relative genetic distances (cM) of the polymorphic markers using MAPMAKER software.

Group	Marker	Genetic distance
1	**LU38**	15.8cM
	6H5	
2	**2D2U**	5.5 cM
	2D2L	
3	**LU15**	26.0 cM
	9B2	
4	**LU31**	15.8 cM
	LU28	7.2 cM
	3B5U	3.2 cM
	7F2	
5	**5B6**	4.9 cM
	8A11	

Phylogenetic analysis

Similarities and dissimilarities of 95 RILs and the two parents reflected by the 19 polymorphic marker loci were calculated and clustered into several groups (Figure 5.4). The 95 RILs were grouped together under two main clusters. The two parents, Viking and E1747, fell into the two different clusters with a genetic similarity of 37 % (Figure 5.4). Several sub-clusters were also observed within these two main clusters.

RIL 293 was most similar to the Viking parent with a genetic similarity (GS) of 95 %, whereas RIL 330 was most similar to the E1747 parent with a genetic similarity of 92 %. The most divergent RILs found in this study were RIL293 and RIL302.

Two out of five highest fibre concentration RILs (350, 264) (see Chapter 3, Table 3.7.1) fell under the Viking cluster while the other three (337, 341 and 306) fell under the E1747 cluster. Four out of the five lowest oil containing RILs (350, 280, 298, 342) (see Chapter 3, Table 3.7.2) fell under the Viking cluster. Interestingly, three out of the five highest oil containing RILs (295, 320 and 291) fell under the Viking cluster. All the five highest linolenic acid concentration RILs (343, 255, 303, 300, 283) fell under the Viking cluster. In addition, three out of the five lowest linolenic acid containing RILs (308, 345, 273, 309) also fell under the Viking cluster.

The potential dual purpose RILs with high fibre concentration, high oil concentration and low linolenic acid concentration (Chapter 3, Table 3.8.1 and 3.8.2) were dispersed along the dendogram with three RILs falling under the Viking cluster (RIL273, RIL287, RIL311) and RIL319 and RIL309 under the E1747 cluster (Figure 5.4).

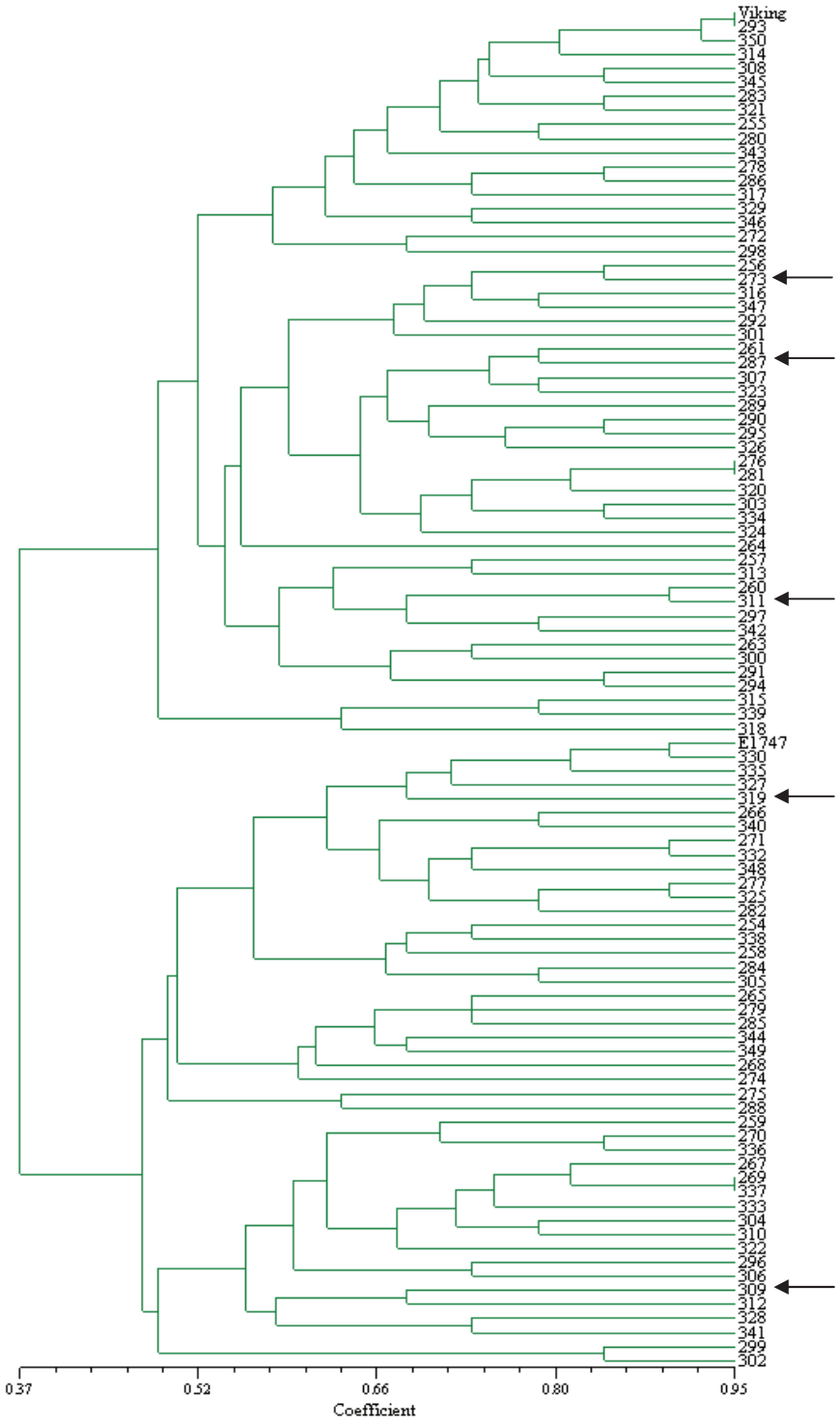


Figure 5.4. UPGMA dendogram based on similarity (17 SSR and 2 CAPS markers) for Viking, E1747 and 95 RILs derived from Viking × E1747. The arrows indicate the position of the potential dual purpose lines.

Discussion

This study focused on identifying molecular markers associated with both stem fibre and oil characteristics. In the past there were few molecular markers associated with fibre or oil traits. In an AFLP based linkage map study conducted by Vroman (2006) using 186, F_9 Viking × E1747 recombinant inbred lines (RIL) population; several QTLs were identified as linked to many agronomically important traits. However, due to the labour intensive and dominance nature of the AFLP markers, the focus of this study was to establish a set of molecular markers that were easy to use and highly reproducible for application in MAS.

The screening of the parents and the RIL population for EST-SSRs available fibre ESTs from GenBank (Day et al., 2005b) revealed a low degree of polymorphism between the parents and the RIL population. This result is supported by earlier reports (Roose-Amsaleg, 2006; Vroman, 2006) and may be due to self fertilization and strong selection by breeders over the years. The percentage of SSRs detected from the EST collections has also been found low in flax (3.5 %) (Cloutier et al., 2009), barley (2.8 %) (Varshney et al., 2006) and wheat (7.4 %) (Peng and Lapitan, 2005). However, the presence of a significant association between some of the polymorphic markers in other EST-SSR collections (Table 5.2) indicates the importance of testing these specific markers for MAS.

Single marker regression analysis

Single marker analysis, also sometimes referred to as single factor analysis or single point analysis, has been used extensively, especially in crops which are deficient in markers or lack a genetic linkage map (Chandra et al., 2004). Choosing an appropriate single-marker association test is critical to the success of genetic association studies. An ideal single-marker analysis should have robust performance across a wide range of environments. In this study, both single marker regression analyses as well as Stepwise Regression Analysis (SWR) were used to identify QTL harboring markers in order to yield accurate and reproducible results. SWR is reported to select fewer markers than SMA, with the least number of false positives and so is one of the potential models to prevent detection of spurious linked markers (Chandra et al., 2004; Murthy et al., 2005).

Fibre related traits

Little is known about the inheritance of fibre content, but the moderate to high previously observed heritabilities (Fouilloux, 1989) suggest that selection for fibre content should be effective (see Chapter 3 , Table 3.4). Furthermore it is suggested that both additive and dominant effects of genes are involved in the heredity of fibre content and both effects are influenced by environmental conditions (Popescu et al., 1998). Molecular studies using AFLP markers suggested that at least four major QTLs were involved in controlling fibre content (Vroman, 2006). In the present study using SMA and SWR, we found that only one marker (LU8) was loosely associated ($p < 0.05$) with fibre concentration (Table 5.2). It is likely that a greater density of SSR markers is needed in order to precisely identify the markers strongly associated with fibre concentration. However, the marker found polymorphic in this study may be of potential value for future marker discovery projects as well as for varietal identification.

Earlier studies on flax plant height have shown high heritability for this trait (Foster et al., 1997). Five plant height QTLs were detected across a RIL population derived from a Belinka × Hermes cross based on AFLP mapping (Vroman, 2006). In the present study, SMA as well as SWR revealed that the two markers, LuFAD3B and CV8824, were significantly associated with plant height ($p < 0.01$) and could be of potential use for selecting tall or short flax cultivars.

Oil related traits

It was interesting to note that two SSR markers, namely 5B6 and 8A11, accounted for 17.2 % ($R^2_{5B6} = 10.4\%$; $R^2_{8A11} = 6.8\%$ in Table 5.2) of the total phenotypic variation for oil concentration using the SMA method and were mapped on the same linkage group (group 5) with a genetic distance of only 4.9 cM (Table 5.3). These two markers along with a third unlinked SSR marker, LU3, explained in total about 24.4 % of phenotypic variation in oil concentration. However, based on the SWR analysis, only 5B6 showed a strong association ($p < 0.01$), confirming its role in influencing oil concentration. In addition, another SSR marker LU32 has been found to be strongly associated with protein concentration, $p < 0.01$) but not found to be linked with any other markers (Table 5.3). Previously, three oil concentration and four protein concentration QTLs were reported across a Viking × E1747, 186 entry RIL population based on AFLP mapping (Vroman, 2006).

Two CAPS markers, LuFAD3A and LuFAD3B, were reported as tagging genes controlling linoleic acid desaturation in flax (Vrinten et al., 2005). The results from this study have confirmed this report as LuFAD3A and LuFAD3B markers together explained 84 % ($p < 0.0001$) of phenotypic variation for linoleic and linolenic acid concentration. Interestingly, LuFAD3A also had an association with palmitic acid concentration (R^2= 20 %, $p < 0.001$) and oleic acid concentration (R^2= 18.5 %, $p < 0.001$) using both SMA and SWR methods. Similarly, LuFAD3B also was strongly associated with palmitic acid (R^2= 7.9 %, $p < 0.01$) and oleic acid % (R^2= 7.4 %, $p < 0.01$) though in lesser magnitude as compared to LuFAD3A (Table 5.2). Together these two CAPS markers accounted for approximately 28 % of phenotypic variation in the concentration of palmitic and oleic acid in flax seed. In a similar study, Vroman (2006) reported the presence of four QTLs for palmitic acid concentration. However a previous study by Ntiamoah et al. (1995) found that palmitic acid concentration of flax seed was controlled by a single additive gene. It might be that as fatty acid biosynthesis follows a linear pathway (palmitic: stearic: oleic: linleic: linolenic) the above mentioned marker associations found in this study were only due to the upstream and downstream chain effects of this pathway. Irrespective of these discrepancies, this study has confirmed the importance of LuFAD3A and LuFAD3B genes/ markers in breeding for specialty fatty acid profiles in flax.

Genetic diversity study

Genetic diversity analysis in RIL populations is important in selecting desirable genotypes based on their genetic similarity/ dissimilarities for use as parents in breeding programs (Liu et al., 2006). The genetic diversity study of the 95 RILs and the two parents, Viking and E1747 revealed the presence of two different clusters with each parent forming the edges of each cluster. Genetic similarity of only 37 % between the parents has confirmed that Viking × E1747 RIL population is a promising mapping population to identify genes / markers related to both fibre flax as well as oilseed flax characters (Vroman, 2006). Most of the low oil concentration (four out of five) RILs belonged to the Viking cluster. However, the dendogram analysis did not allow the separation of the RILs into groups based on other phenotypic traits (see Chapter 3) such as fibre, plant height and other oil related traits (Figure 5.4). This shortcoming may be due to the production of intermediate types with respect to the marker/phenotypic data, which are segregating for both fibre as well as oil traits. This suggestion

is supported by the observation that the potential dual purpose lines (RILs- 311, 319, 273, 309 and 287) were found distributed along both Viking and E1747 clusters indicating that they inherited genes from both the parents (Figure 5.4).

The number of markers used can affect the variance in similarity estimate because a marker represents an independent genomic sample (Powell et al., 1996a,b). The distribution of markers in the genome is also an important factor to consider in diversity studies as a good coverage of the genome improves its representation efficiency and the strength of comparison between individuals. However, it is normally assumed that markers are randomly distributed in the genome (William et al., 1990; Bonato et al., 2006). Previous research indicates that SSRs provided a wide coverage of the plant genome and is evenly distributed across the genome (Powell et al., 1996b). Further marker discovery attempts are being carried on (McKenzie et al., 2008; Cloutier et al., 2009, 2010; Soto-Cerda et al., 2011a, b; Bickel et al., 2011; Deng et al., 2011) in order to improve the reservoir of molecular resources in flax. Recently, a set of 248 SSR markers was found polymorphic on 23 flax accessions derived from 146,611 ESTs from flax plantlets during first branching stage (Cloutier et al., 2008, 2009).

In conclusion, molecular marker assisted selection seems to be a viable exercise in obtaining genotypes with improved performance in both oil and fibre related characters. This study has identified some important, easy to use markers that can be effectively used in marker assisted selection for improving many agronomically important traits. The markers reported here are highly consistent over different environments. The study has confirmed the suitability of the Viking × E1747 RIL population as a potential mapping population for dual purpose traits improvement. However, further molecular marker studies would be needed to accurately map the genes of interest in order to improve both oil and fibre characteristics in flax.

CHAPTER 6

Microarray analysis of bast fibre producing tissues of fibre and oilseed type flax (*Linum usitatissimum* L.)

Abstract

In this study, global transcript profiling using cDNA - based microarrays was performed to identify differentially expressed fibre related transcripts between fibre flax (Viking) and oilseed flax (E1747) genotype at the seedling stage. A 9600-clone cDNA library from the fibre rich phloem bearing tissues of flax was analyzed. It was interesting to find that the largest group (7 %) of transcripts abundant in Viking fell under the functional group involved with cell wall development. Some of the interesting transcripts abundant in Viking relative to E1747 included callose synthases, cytochrome P450, fasciclin like arabino galactan proteins and β - galactosidases. The transcripts more abundant in E1747 relative to Viking included UDP – glucose glucosyltransferase, auxin repressed protein, ubiquitin conjugating enzyme, peroxidases and lipid transfer proteins. The quantitative real time PCR results confirmed the suitability of the microarray platform to accurately discriminate transcript profiles between the two diverse flax types. Identification of these candidate genes will further tailor the application of both classical and molecular breeding towards expanding the knowledge on fibre biosynthesis in flax and other bast crops.

Introduction

Flax (*Linum usitatissimum* L.) is a self-pollinating annual crop grown for its stem fibre or seed oil. Flax varieties grown for seed oil (oilseed) have shorter main stems with multiple branches and seed capsules whereas fibre flax varieties have long, slender and unbranched stems (Hayward, 1948). The overall bast fibre content of the oilseed flax stems ranges from 11-15 % (Burton, 2007) while that of fibre flax varieties ranges from 17-35 % (Booth et al., 2004). Resurgence of fibre flax for production of natural fibres, as well as already established markets for seed oil raises, the question whether the same plant can be used for both purposes. A number of studies have been conducted to understand the genetics of seed oil production in flax (Green

and Marshall, 1981; Rowland, 1991; Ntiamoah et al., 1995) but unfortunately, information about the stem fibre related genes is not clear.

During plant growth, fibres develop in two main steps namely: cell elongation and thickening of secondary walls. Although cellulose is the main component in the mechanical properties of a fibre cell wall, the cell wall formation and development is a highly complex and dynamic process involving many other genes related to cell wall remodelling and expansion. Carpita et al. (2001) calculated that 15% (3800) of Arabidopsis genes are directly involved in cell wall metabolism. It is assumed that the transition between cell elongation and cell wall thickening of these unique fibre cells in flax occurs at the "snap point" region (Gorskova et al., 1996). The stem region above the snap point consists of fibres undergoing elongation, the region surrounding the snap point contains fibres that are in transition between elongation and cell wall development, and below the snap point, fibres undergo secondary cell wall deposition.

Several molecular approaches have been pursued in order to better understand the processes involved in xylem and phloem differentiation in vascular plants, one of the latest being the use of microarrays. A microarray is a tool for analyzing gene expression on a small membrane or glass slide containing samples of many genes arranged in a regular pattern (NCBI, 2007). Microarrays thus provide a high throughput tool in studying the temporal expression patterns of thousands of genes in a single experiment quickly and efficiently. There have been several reports of microarray studies on cotton fibre development especially the early stages of fibre initial formations in the growing cotton ovule (Ji et al., 2003; Xu et al., 2007; Taliercio and Boykin, 2007). Studies on cotton fibre cell development showed complex processes involving many pathways, including signal transduction, transcriptional regulation components and phytohormonal regulation (Lee et al., 2007).

With regard to studying plant vascular development, Demura et al. (2002) used oligonucleotide – based microarrays to identify genes associated with secondary cell wall biosynthesis of *Zinnia elegans* cell cultures undergoing tracheary element differentiation. Hertzberg et al. (2001) used poplar cDNA arrays to profile changes in gene expression at various stages of secondary xylem differentiation. Oh et al. (2003) and Ko et al. (2004) used oligonucleotide arrays to identify genes expressed during secondary xylem growth in Arabidopsis. Birnbaum et al. (2003) used microarrays to profile the developmentally regulated

expression of genes in Arabidopsis root tissues. However, very few researchers working on vascular biology have concentrated on phloem tissues, particularly bast fibres. Recently, De Pauw et al. (2007) studied transcript profiling of hemp phloem tissues, whereas Roach and Deyholos (2007) studied flax phloem tissues by comparing gene expression in different vertical stem sections. Some of the interesting transcripts that were highly expressed in the flax study were those of lipid transfer proteins (LTPs), arabinogalactan proteins (AGPs), chitinases and β-galactosidases.

Studies on understanding the molecular processes between contrasting flax types such as fibre and oilseed flax with respect to development of bast fibre are very limited (Fenart et al., 2011). In order to better understand and compare the differential transcripts present in each of these diverse flax types, a microarray experiment was conducted using the fibre bearing phloem tissues from Viking (fibre flax) and E1747 (oilseed flax) genotypes. Viking is a European fibre cultivar possessing high stem fibre concentration and desirable plant height for use in the textile industry. E1747 on the other hand, is an ethylmethane sulphonate (EMS) mutant line from McGregor, a Canadian oilseed flax variety with good oil characteristics including < 5 % linolenic acid content, which makes it highly suitable for commercial edible-oil markets. These two genotypes provide a unique system to compare the mechanisms of fibre cell differentiation, elongation and thickening between the two diverse flax types. Interestingly, due to the diverse nature of these two genotypes, Viking × E1747 derived recombinant inbred lines (RILs) has also been the choice of other molecular genetics studies in flax for oil improvement (Klocke, 2000) as well as understanding the genetic diversity and linkage mapping in flax using AFLP molecular markers (Vroman, 2006).

The objective of this study was to compare and identify genes/ transcripts that contribute to the pattern of fibre production in fibre flax as compared to their oilseed counterpart. In order to avoid sampling biases because of fibre type flax depositing more fibre as compared to the oilseed types, samples were taken at an early stage of plant growth (2-3 weeks after germination). Young seedlings and hypocotyls have also been commonly used model to study primary growth in plants due to rapid cell elongation during this stage (Ji et al., 2003; Roach and Deyholos, 2007, 2008)

The RNA from phloem bearing tissues in this study was extracted from below the snap point region of the flax stem due to the ease in peeling off the phloem rich bast tissue. It is worth mentioning that the microarray may miss the transcripts involved at the meristamatic region and the snap-point region (such as cell elongation, cell differentiation and remodelling), and may pick up transcripts involved in cell wall deposition/ thickening due to sampling from this stem region. However, as the flax seedlings undergo fast stem elongation during the early vegetative growth stages coinciding with the sampling period (Gorskova et al., 2003), it is also anticipated that some specific transcripts involving rapid cell elongation and differentiation in addition to those involved in secondary cell wall thickening might still be expressed in the stem peels extracted from the below snap point region of the flax stems.

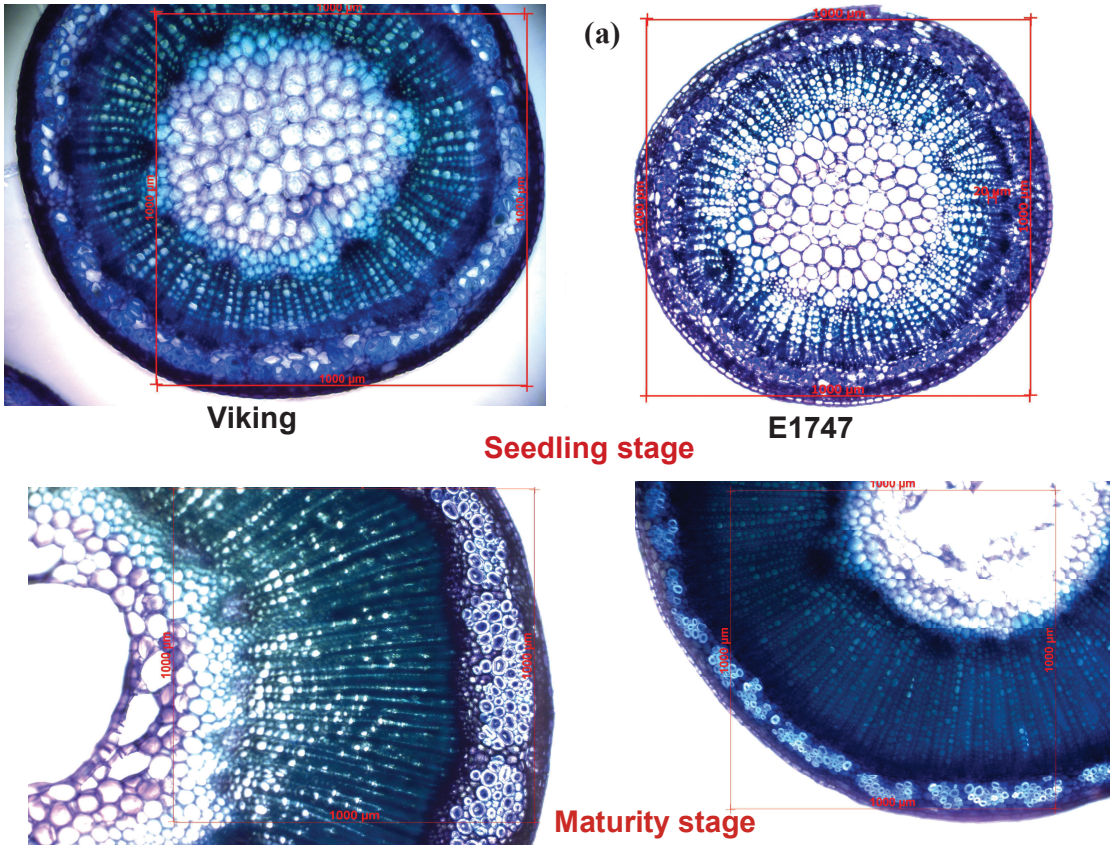
Materials and methods:

Plant growth conditions

Plants were grown in the Phytotron at the University of Saskatchewan, Saskatoon during the month of September, 2007. Seeds of *Linum usitatissimum* L. fibre flax variety Viking and oilseed variety E1747 were raised at 25^{o}C day and 17^{o}C night on an 18 h light / 6 h dark cycle. Plants were fertilized at sowing with 15-30-15 all purpose N-P-K fertilizer (Plant Prod, Bramton, ON, Canada) according to the manufacturer's instruction in Sunshine Mix G3 soil. Images of both the flax genotypes Viking and E1747 are depicted in Figure 6.1.

RNA extraction

Two to three-week old flax seedlings (days after germination) measuring 15-20 cm in height were harvested for this study. The outer stem layers (stem peels) comprising the epidermis, cortex, phloem cells and the cambial region (Figure 6.2) were extracted from below the snap point region and immediately frozen in liquid nitrogen. At least 10 plants were pooled and used for RNA extraction with the RNAeasy Plant Mini kit (Qiagen, German-town, MD) following manufacturer's directions.



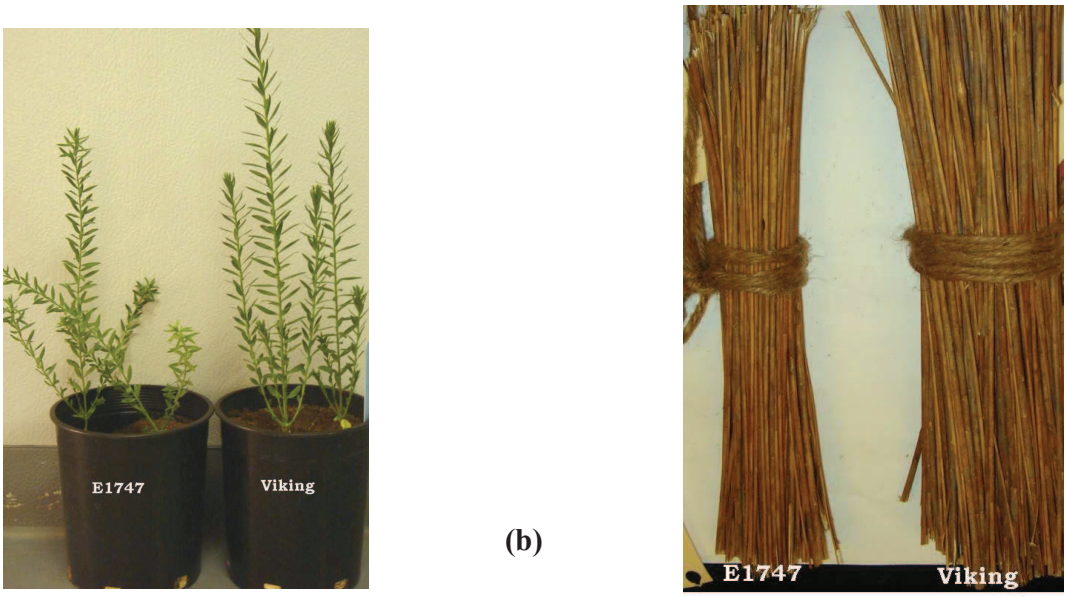


Figure 6.1. a.) Comparative anatomical stem cross-sections of the two diverse genotypes (fibre flax: Viking and oilseed flax: E1747) at seedling and physiological maturity stages stained with Toluidine Blue O (0.1 %) using optical microscope at 10 x magnification. b.) Flax seedlings grown at the phytotron and straw bundles harvested at physiological maturity stages from 3.7 m long field rows of Viking and E1747.

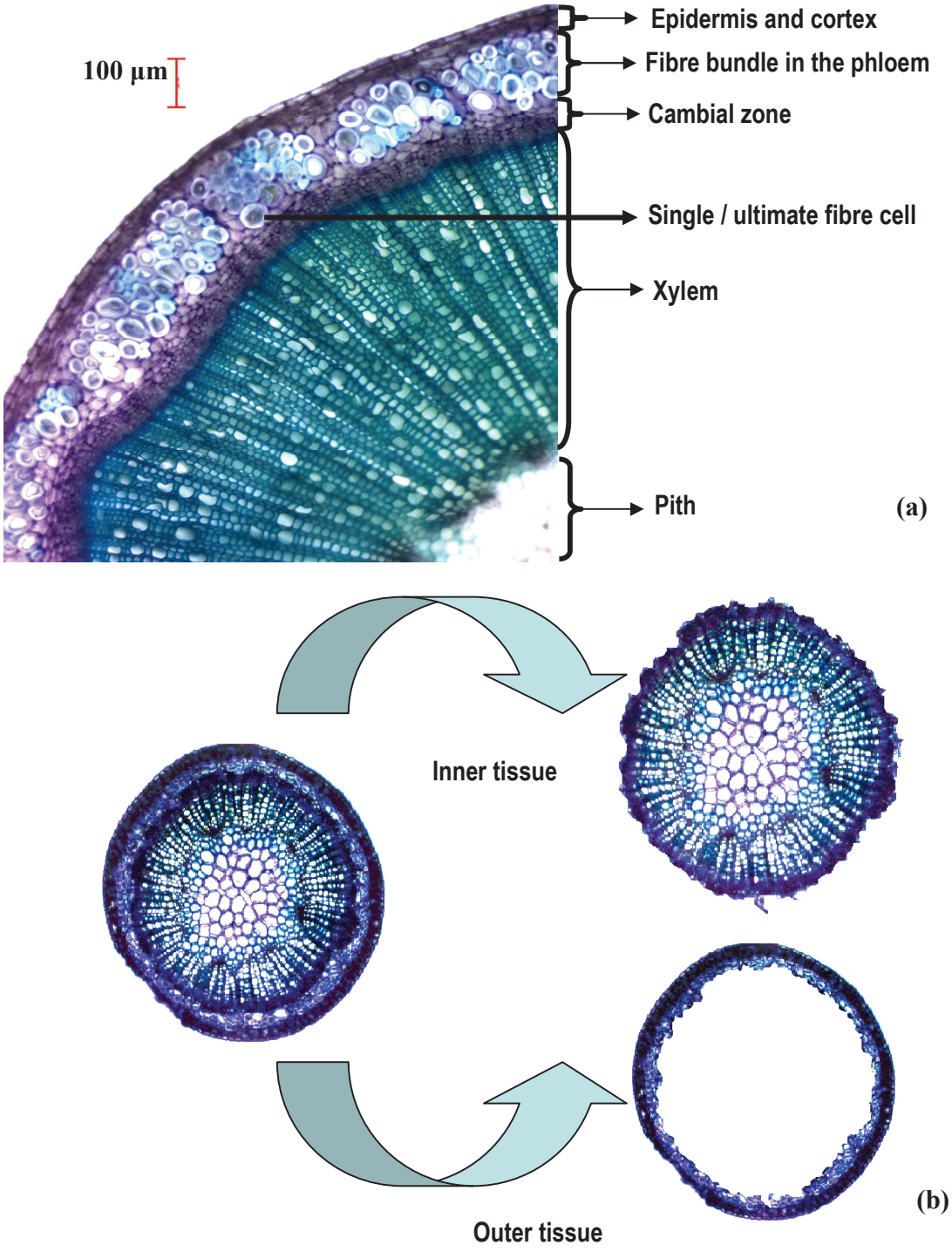


Figure 6.2. a.) Photomicrograph of flax stem (Viking) with labeling of different anatomical segment. b.) Tissue arrangement in "fibre – rich stem peels" containing epidermis, cortex, phloem cells and cambial tissues used to extract RNA for the microarray experiment.

Microarray methods

A cDNA library and microarray amplicons were constructed at the University of Alberta, using the oilseed flax variety Norlin. Methods for printing PCR-amplified cDNA inserts onto high density microarray glass slides have been described by Roach and Deyholos (2007). To summarize the procedure, the PCR amplicons were purified using Montage $PCR_{\mu 96}$ 96-well plates (Millipore, Billerica, MA), concentrated by vacuum manifold and resuspended in 30 µL of sodium phosphate buVer (30 mM, pH 8.6). The samples were then concentrated to 6 µL for spotting on SuperAmine microarray slides (Telechem, Sunnyvale, CA) using an Omnigrid 100 robot (Genomic Solutions, Ann Arbor, MI) following the manufacturer's directions.

A total of 9,600 amplicons were spotted once in each of two duplicated arrays on each slide. cDNA derived from stem peels of both the flax genotypes (Viking and E1747) were synthesized using SuperscriptII (Invitrogen) and primed with RT polyA-capture oligomers from the 3D Array900 microarray labeling kit (Genisphere, Hatfield, PA). Both samples were hybridized together on the microarray slide at 60 °C overnight under a 24 × 60 mm coverslip in a sealed humid 50 mL centrifuge tube. Secondary hybridization with Cy3 and Cy5 dendrimers (Amersham Biosciences) were performed according to the manufacturer's protocol, and slides were scanned immediately using an ArrayWorxc scanner (Applied Precision, Seattle, WA). In order to avoid any dye bias, which may affect the labeling efficiency and/or differences in recording fluorescent signals, dyes were swapped for each hybridization. Three independant biological replicates of the comparison were conducted for a total of six-dual color hybridizations/ replications. A general schematic representation of the microarray experiment is depicted in Figure 6.3.

Microarray data analysis

Annotation of flax EST sequences used in the microarray slides were obtained from the University of Alberta in collaboration with Dr. Michael Deyholos and his colleagues (Roach and Deyholos, 2007). The microarray spot intensities were quantified using Spotfinder v3.0 (Saeed et al., 2003). Typically the intensity ratios are log transformed (logarithm base 2 is most widely used). The advantage of this transformation is that up-regulated and down-regulated values are comparable and of the same scale (Quackenbush, 2002; Leung and Cavalieri, 2003). This means

that an up-regulated gene with intensity ratio = 2 has a $\log_2$ (ratio) = 1, while a down-regulated gene with intensity ratio = 0.5 has a $\log_2$ (ratio) = -1, and a constantly expressed gene with intensity ratio = 1 has a $\log_2$ (ratio) = 0. For the purpose our microarray calculations all cDNA clones that had signal intensity ratios greater than zero represented greater abundance of transcripts in Viking relative to E1747 (depicted in red color) while cDNA clones with signal intensities less than zero or negative represented greater transcript abundance in E1747 relative to Viking (depicted in green color).

After the image processing, but before the downstream analysis of the gene expression profiles, the relative fluorescence intensities in each of the scanned channels was normalized. The goal of normalization was to identify and overcome as much of the technical variation as possible such that the observed signal intensity ratios most accurately reflected the biological variance. The normalization of microarray data was performed by the implementation of the Lowess algorithm using MIDAS v2.19 software (Saeed et al., 2003) (Figure 6.4). Significance analysis of microarrays (SAM) was applied to find spots for which the fold change differed significantly between both genotypes using a false discovery rate (FDR) of 5 % (Tusher et al., 2001) (Figure 6.5). Cluster analysis was used to more systematically group related gene expression patterns across experiments. The unsupervised hierarchical clustering analysis (HCL) using average linkages were used to group the clones with similar signal intensities and produce a dendogram (Eisen et al., 1998) (Figure 6.6 and Figure 6.7).

Functional categories or biological processes were assigned to the differential expressed transcripts based on Gene Ontology (GO) annotation (http://www.geneontology.org March 10th, 2008) (Table 6.3 and Appendix Table D.1).

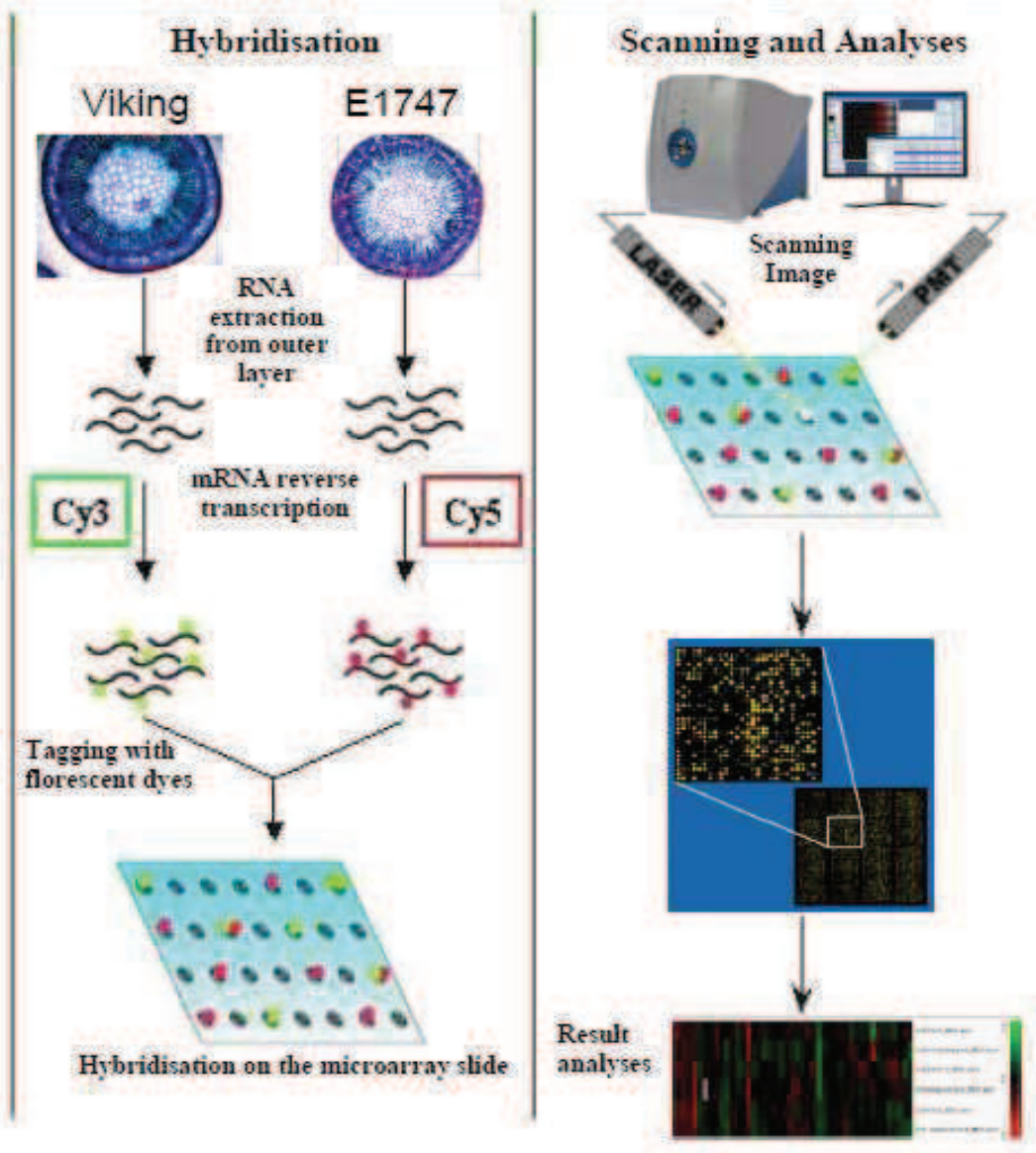
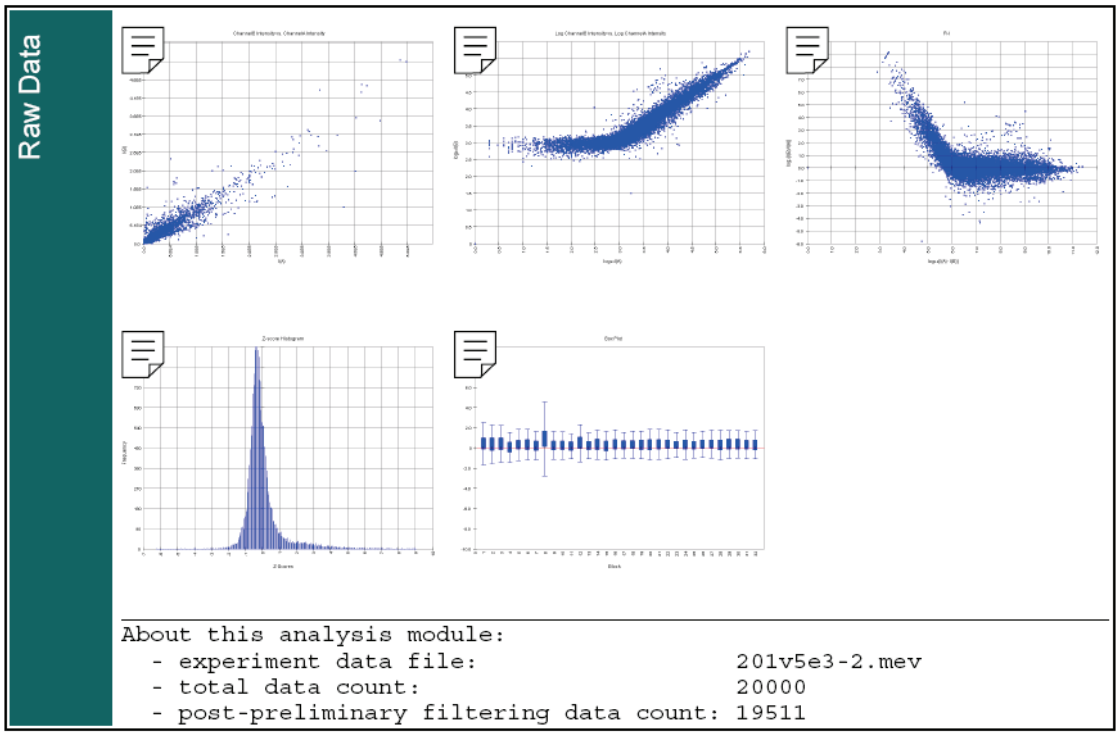


Figure 6.3. General schematic representation of microarray experiment starting from RNA extraction, tagging of fluorescent dyes, hybridization on the microsrray slide, imaging and result delivery by microarray data analysis. (Modified from Crom, 2003)



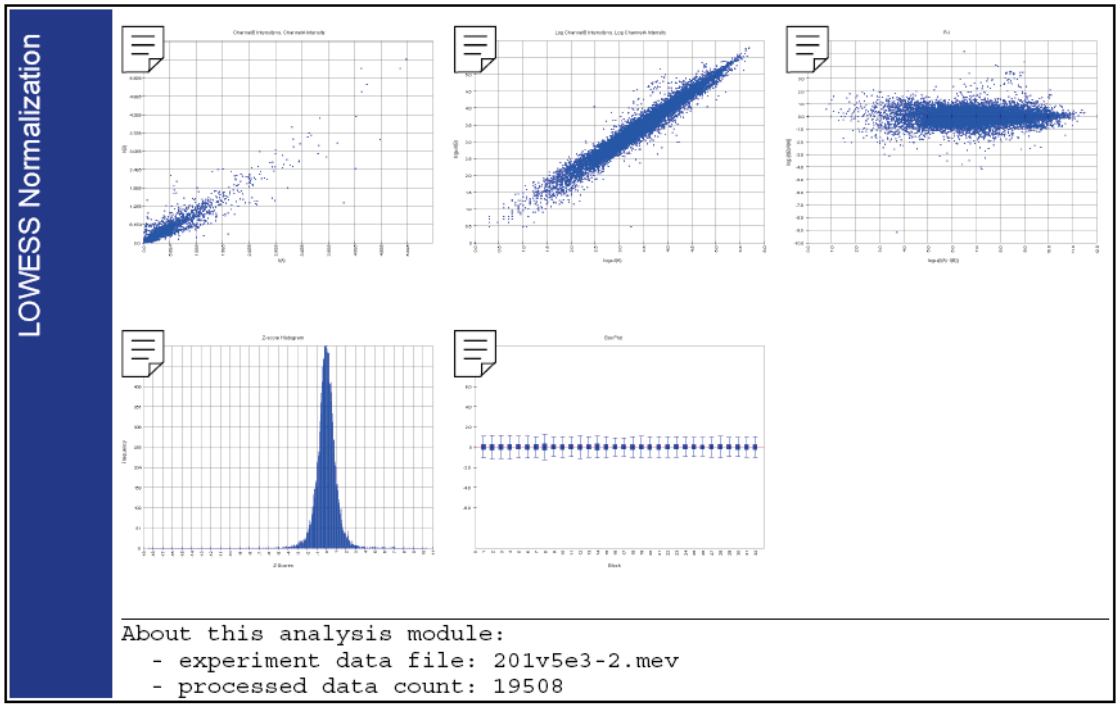


Figure 6.4. Effect of Lowess normalization on flax microarry data. The top panel represents un-normalised microarray data, the bottom panel represent the normalized date using non-linear locally weighted scatterplot smoothing (Lowess) regression analysis performed by Microarray Data Analysis Software (MIDAS) v2.19 software (Saeed et al., 2003).

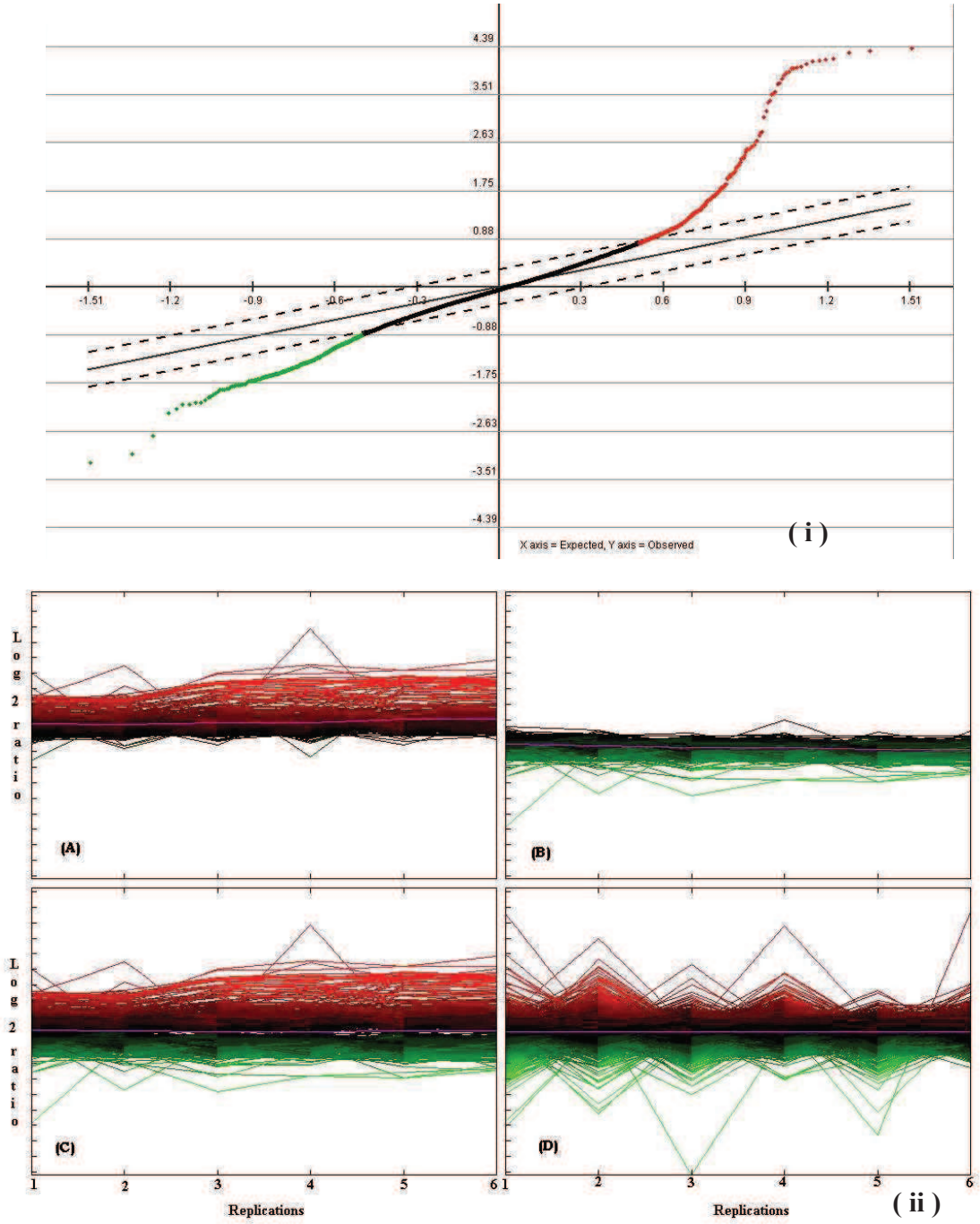


Figure 6.5. (i) The graph represents the filtering of microarray spots for which the fold change differed significantly between two genotypes of flax. Significance analysis of microarrays (SAM) was applied to find spots with a false discovery rate (FDR) of 5 % (Tusher et al., 2001). Red spots or lines correspond to transcripts being more abundant in the Viking genotype as compared to E1747 and green spots or lines represents vice-versa. (ii) The graphs represent the significant transcript expressed in each of the genotypes (A-Viking and B- E1747), (C) Represents the total of all the significant transcripts filtered using SAM analysis (D) Represents all the non-significant genes that were filtered out using the SAM analysis over six replications.

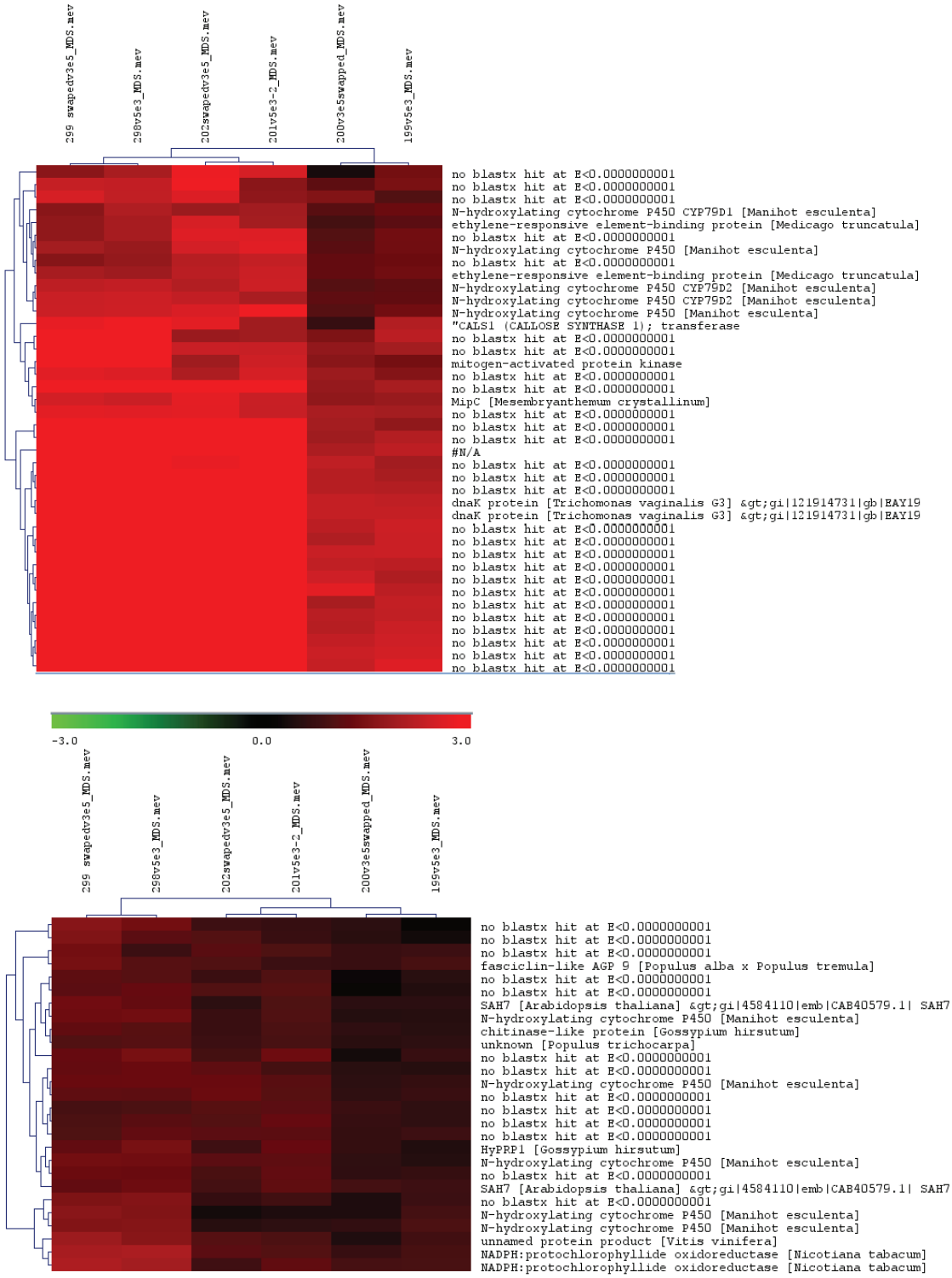


Figure 6.6. Hierarchial cluster analysis (HCL) of the significant transcripts (including duplicates) found more abundant in Viking relative to E1747 based on mean expression ratios (log_2 Viking/E1747) using average linkage method over six hybridizations/ replications.

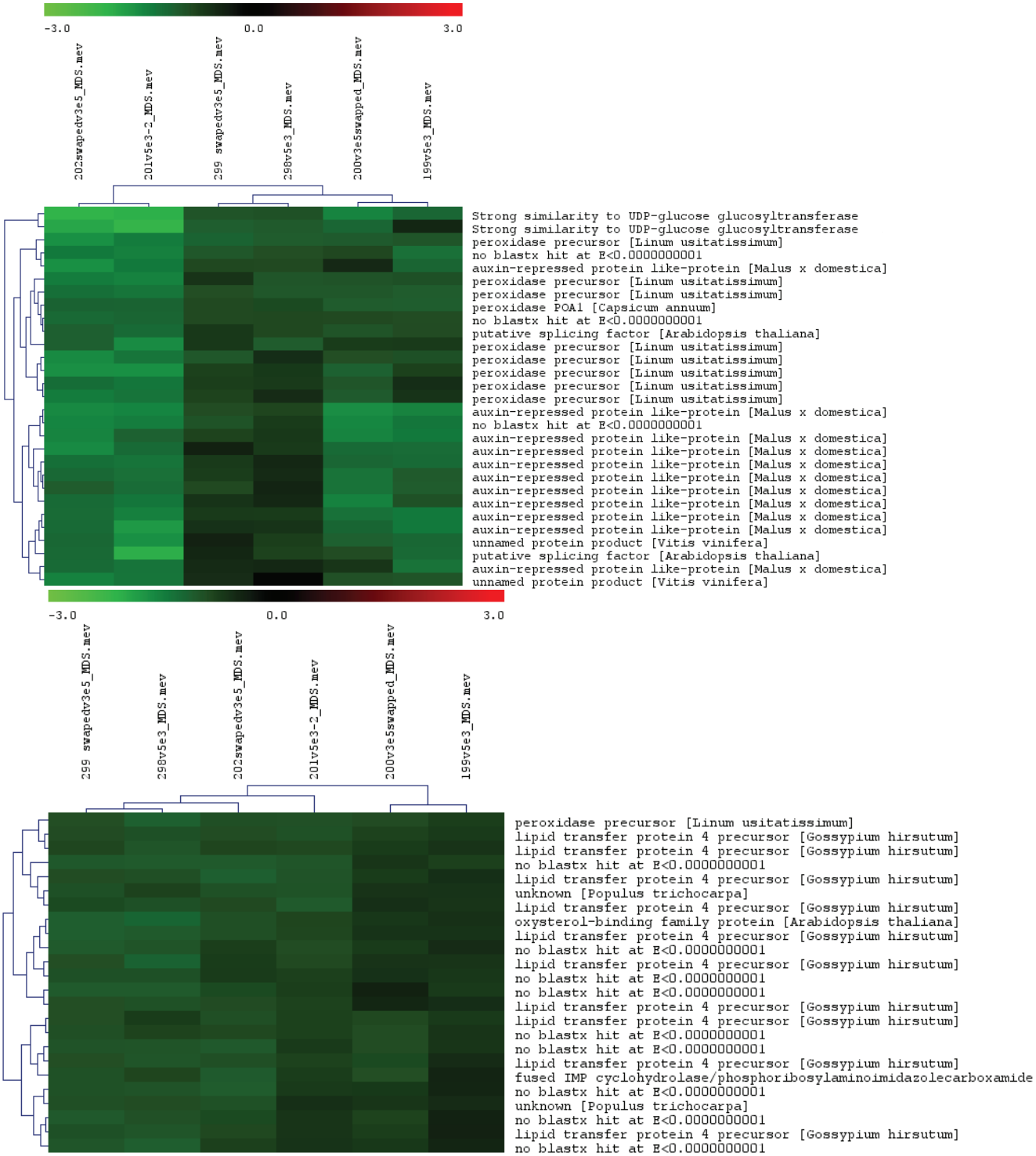


Figure 6.7. Hierarchial cluster analysis (HCL) of the significant transcripts (including duplicates) found more abundant in E1747 relative to Viking based on mean expression ratios ($\log_2$ Viking/E1747) using average linkage method over six hybridizations/ replications.

Quantitative real-time PCR (qRT-PCR)

Aliquots of three biologically independent RNA samples with three technical replications were used for real-time PCR to test six different transcripts (three more abundant in Viking and three more abundant E1747). cDNA from stem peels of flax was extracted using Quantitect reverse transcription kit (Qiagen) from 1 μg of total RNA using manufacturer's directions. Real-time PCR was performed in an Applied Biosystems 7500 Fast Real-Time PCR system using SYBR Green. For quantitative PCR reactions, 2.5 μL of a one-sixty fourth dilution of the reverse transcription reaction was used in a total volume of 10 μL with 0.8 μM of each forward and reverse gene-specific primer, 0.2 mM dNTP, 1x ROX, 0.25x SYBR green and 0.03U / μL Platinum *Taq* (Invitrogen). The PCR cycles were conducted as described in Roach and Deyholos, (2007). Threshold cycles (C_T) were determined using 7500Fast Software. C_T values were normalized using elongation factor 1α (EF1a) as an endogenous control. ddCT values were generated using Viking sample as reference and $\log_2$ ratios calculated to show relative expression values between Viking and E1747 fibre related tissues. The primer sequences for the six selected transcripts used in the qRT-PCR analysis are depicted in Appendix D.

Results

Microarray analysis

In the microarray experiment, a 9600-clone cDNA library derived from the phloem bearing tissues of Norlin oilseed flax were used as the target. Out of 9,600 cDNA clones 7,716 had microarray hybridization. The SAM algorithm revealed that only 1,198 clones had microarray hybridization signal intensities significantly different between the two parents with a FDR < 5 % (Tusher et al., 2001) (Figure 6.5). The signal intensities of these 1,198 clones were re-calculated and duplicates were averaged over six replications resulting in 799 clones with significantly different expression.

The HCL clustering was based on similarity in signal intensities and included the duplicates of a clone. The most abundant transcripts clustered in both Viking (red panel) and E1747 (green panel) are depicted in Figures 6.6 - 6.7.

Out of these 799 clones, 537 clones were picked randomly in order to perform high quality sequencing. The clones with high sequence similarity (E - value $\leq 10^{-10}$) were aligned together and grouped into contigs while the rest were considered as singletons. This operationally unique set of clones, which constitute a contig or a singleton, will be referred to hereon as a "probe set". Thus, each of these probe sets may operationally be defined as a unique gene, although further sequence information would be needed to be more conclusive. In total 591 unique probe sets were identified with significant intensity ratios in this study. Out of these 591 unique probe sets 315 displayed greater transcript abundance in Viking as compared to E1747 while 276 probe sets had greated transcript abundance in E1747 relative to Viking.

Microarray comparisons of stem peels

The most abundant sequenced probe set found in the Viking parent (probe set # 102) showed ≥ 3 fold transcript abundance ($\log_2$ ratio = 3.10) relative to E1747. The most abundant probe set found with good sequence quality in E1747 was probe set # 1527 and had a transcript abundance of ≥ 1.6 fold ($\log_2$ ratio = -1.61) relative to Viking. However, both these probe sets did not have any known sequence hits when BLASTx was performed.

Twelve broad functional categories were used to classify differentially expressed transcripts based on the 591 unique probe sets found with GO annotation (http://www.geneontology.org, March 10th, 2008). The functional classifications were photosynthesis, primary energy and metabolism, signaling and gene regulation, transporter, amino acid and protein metabolism, cell wall development, lipid metabolism, nucleotide biosynthesis, oxidoreductases, other biosynthesis, unclassified transcripts and no BLAST hit. Out of the total probe sets taken for classification, 55 % of the probe sets were sequenced. Out of these 55 % probe sets, 28 % did not align with any known sequences, 2 % were unclassified transcripts while 26 % could be categorized into known functional categories (Figure 6.8).

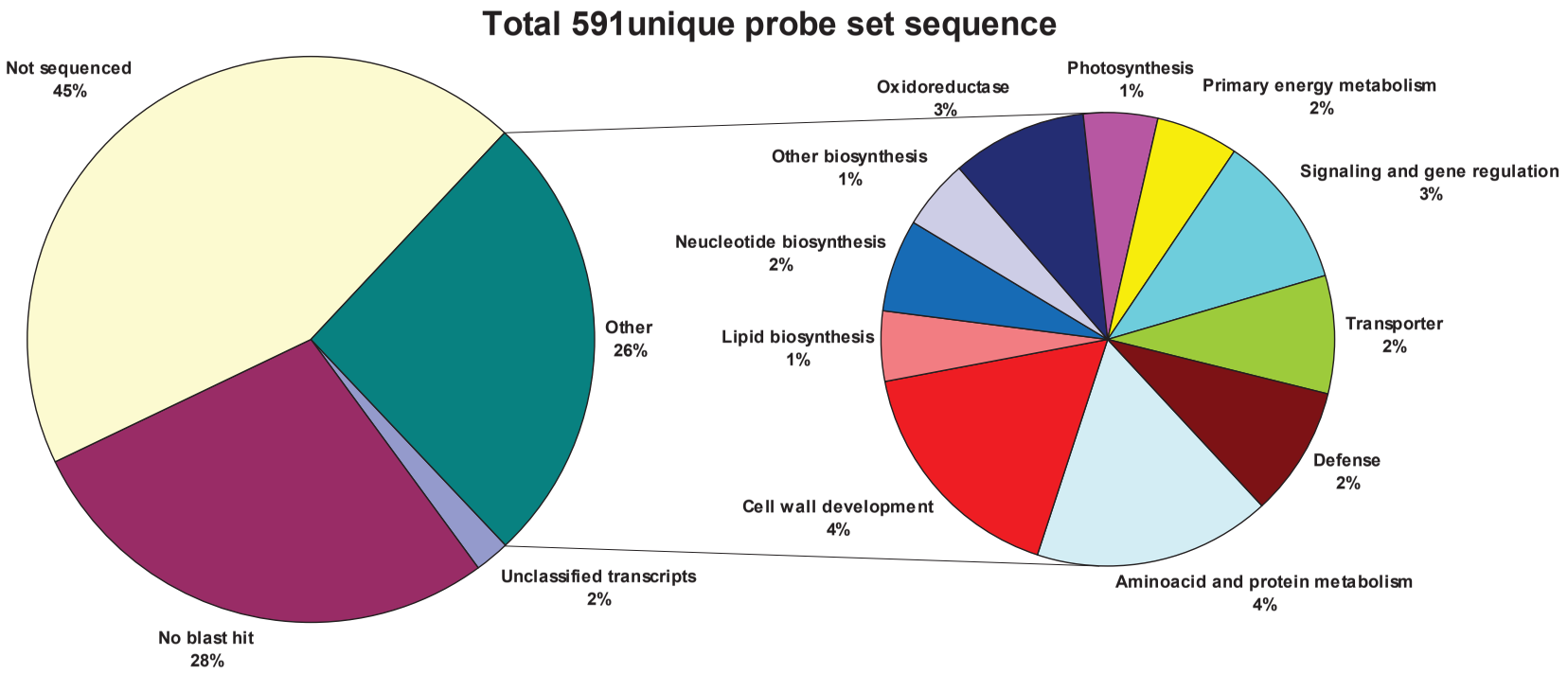


Figure 6.8. Functional classification of all unique probe sets/ transcripts found significant using significance analysis of microarrays (SAM) analysis with a false discovery rate of ≤ 5 %.

Transcripts highly expressed in fibre flax genotype (Viking)

About 315 unique probe sets were found to have more transcript abundance in Viking relative to E1747. However, all the transcripts that had ≥ 2 fold transcript enrichment (log_2 intensity ratio ≥ 2.00) in Viking did not align to any known sequences except one with a BLAST hit for dnaK protein (*Trichomonas vaginalis* G3) (probe set # 259). This probe set # 259 had 12 other clones but none of them had any known BLAST hits indicating ambiguity in the sequence. The other known enriched transcripts in Viking were major intrinsic proteins (MipC), callose synthase, expansin, ethylene responsive element binding protein, mitogen activated protein kinase (MAPK), N-hydroxylating cytochrome P450 and arabinogalactan protein (AGP). The 25 most enriched transcripts in the fibre flax genotype are listed in Table 6.1.

Out of the 315 unique probe sets, 50 % were sequenced and out of these 26 % had transcripts with known BLAST hits in the NCBI Genbank (http://www.ncbi.nlm.nih.gov/genbank/, March 10th, 2008). The most abundant known transcripts in the fibre genotype belonged to the functional categories of cell wall development (7 %), amino acid and protein metabolism (4 %) and oxidoreductase (4 %) (Figure 6.9).

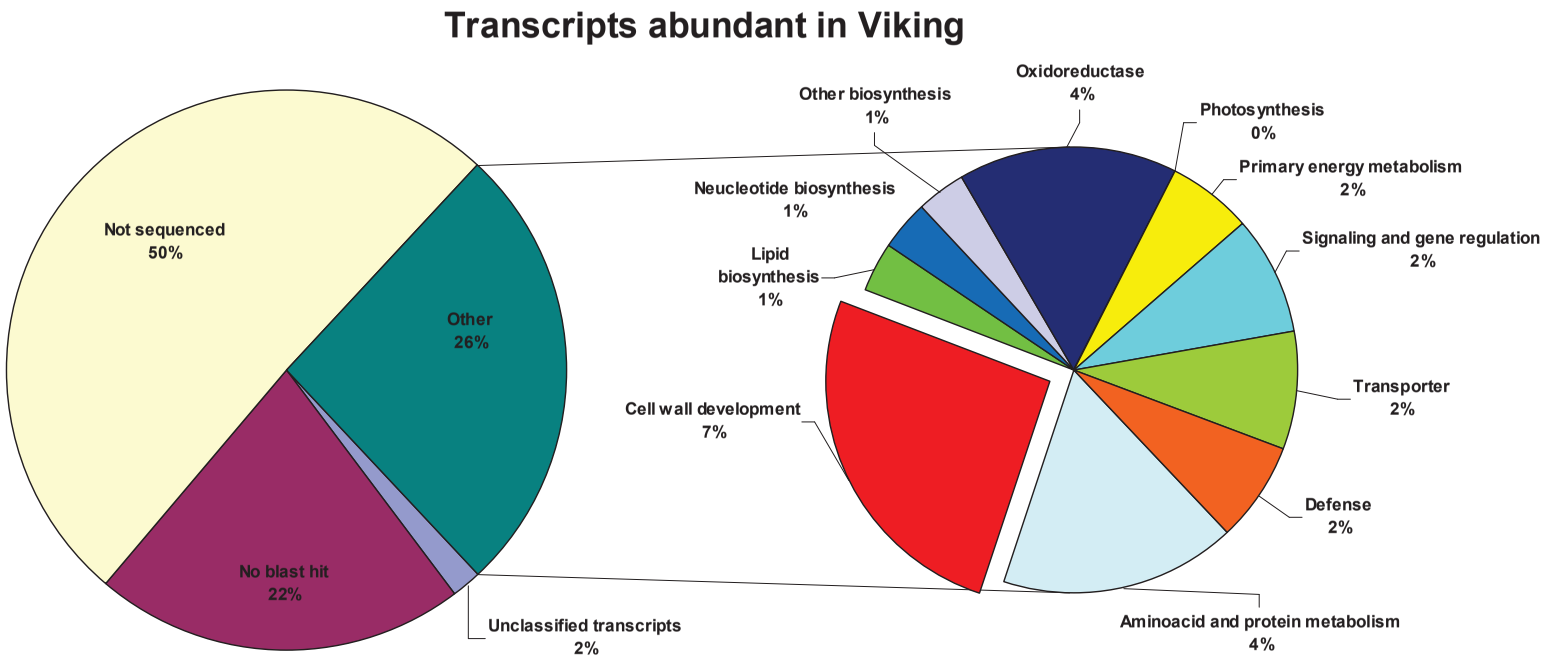


Figure 6.9. Functional classification of all unique probe sets/ transcripts found significant using significance analysis of microarrays (SAM) and more abundant in Viking relative to E1747 based on fold enrichment > 0.00 (log_2 signal intensity ratios - positive) with a false discovery rate of ≤ 5 %.

Table 6.1. List of 25 most abundant transcripts found in the fibre flax genotype (Viking) as compared to oilseed flax (E1747).

Sl. No.	Probe set No.	UID	BLASTx annotation at $E \leq 10^{-10}$ in NCBI database	Signal intensity ratio (Log_2 V / E)	*p*- value	Function
1	102	9322	No BLAST hit	3.10	5.38×10^{-07}	Unknown
2	259	8469	DnaK protein	2.66	$2.34x\ 10^{-10}$	Defense
3	4010	9765	MipC	1.95	3.97×10^{-08}	Transporter
4	7926	304	Callose synthase 1	1.94	$2.26x\ 10^{-05}$	Cell wall development
5	6100	1428	Expansin	1.93	$3.2x\ 10^{-04}$	Cell wall development
6	1556	6739	Ethylene-binding protein	1.75	$1.15x\ 10^{-07}$	Cell wall development
7	665	6001	N-hydroxylating cytochrome P450	1.74	$1.09x\ 10^{-08}$	Oxidoreductase
8	8244	2963	Mitogen-activated protein kinase	1.50	$1.4x\ 10^{-04}$	Signaling & gene regulation
9	2316	851	Arabinogalactan protein	1.30	$1.18x\ 10^{-10}$	Cell wall development
10	7460	2753	Receptor-like protein kinase	1.25	$2.82x\ 10^{-08}$	Signaling & gene regulation
11	1546	4288	Ubiquitin fusion protein	1.24	$1.1x\ 10^{-03}$	A.A & protein metabolism
12	4341	2285	Acetohydroxy acid isomeroreductase	1.20	$3.66x\ 10^{-08}$	A.A & protein metabolism
13	7683	7792	Ketol-acid reductoisomerase	1.19	$3.1x\ 10^{-08}$	Oxidoreductase
14	120	5541	Protochlorophyllide reductase	1.16	$2.51x\ 10^{-06}$	Photosynthesis
15	4342	9781	Dihydropyrimidinase	1.11	$2.4x\ 10^{-06}$	Nucleotide biosynthesis
16	5171	6256	GSDL-motif lipase	1.10	$3.94x\ 10^{-07}$	Lipid metabolism
17	7179	2687	ARR response regulator protein	1.10	$9.39x10^{-09}$	A.A & protein metabolism
18	7120	5102	HyPRP1	1.08	$4.99x\ 10^{-05}$	Cell wall development
19	374	8438	ATP:citrate lyase	1.07	$1.25x10^{-07}$	Lipid metabolism
20	739	4561	Beta-galactosidase	1.03	$3.95x10^{-06}$	Cell wall development
21	224	7840	Fasciclin-like AGP	1.01	$3.59x10^{-08}$	Cell wall development
22	89	2987	Galactose- binding protein	1.00	0.047	Cell wall development
23	2851	4567	Heat shock protein	0.93	$2.19x10^{-06}$	Defense
24	5216	1258	ATP-binding protein	0.90	$1.2x10^{-04}$	Transporter
25	124	977	SAH7	0.89	$1.41x\ 10^{-07}$	Other biosynthesis

Transcripts highly expressed in oilseed flax genotype (E1747)

A number of transcripts have been found to be more abundant (276) in E1747 relative to Viking. The most highly abundant transcript with a fold enrichment ≥ 1.6 (log_2 intensity ratio = -1.61) in E1747 had no known BLAST hit. The next most abundant transcript detected was ubiquitin conjugating enzyme with fold enrichment ≥ -1.3. Some of the other significant transcripts that were found more abundant in the oilseed genotype were UDP-glucose glucosyltransferase, auxin repressed protein, peroxidase precursor and lipid transfer proteins. A list of 25 most abundant transcripts in E1747 relative to Viking has been tabulated in Table 6.2.

Out of the 276 unique transcripts highly expressed in E1747, approximately 64% were sequenced. However, only 26 % of them were found to have known BLAST hits in the NCBI Genbank while 2 % of them were unclassified transcripts. A total of 34 % sequenced transcripts had no known sequence homology. The abundant transcripts in the oilseed parent fell into the major functional categories of amino acid and protein metabolism; signalling and gene regulation; and photosynthesis (Figure 6.10).

A list of all differentially expressed transcripts between Viking and E1747 as represented by GO biological processes / functional categories along with their fold enrichment are depicted in Table 6.3 and Appendix D.

Table 6.2. List of 25 most abundant transcripts found in the oilseed flax genotype (E1747) as compared to the fibre flax genotype (Viking).

Sl. No.	Probe set No.	UID	BLASTx annotation at $E \leq 10^{-10}$ in NCBI database	Signal intensity ratio (Log_2 V / E)	*p*- value	Function
1	1527	5358	No BLAST hit	-1.61	$9.9x10^{-08}$	Unknown
2	4654	996	Ubiquitin conjugating enzyme	-1.33	$2.57x10^{-06}$	A.A & protein metabolism
3	6015	6362	UDP-glucose glucosyltransferase	-1.25	$2.12x10^{-06}$	Other biosynthesis
4	801	1754	Initiation factor 3g	-1.17	$1.69x\ 10^{-06}$	A.A & protein metabolism
5	5954	6404	Putative splicing factor	-1.03	$1.73x\ 10^{-06}$	Nucleotide biosynthesis
6	99	874	Auxin-repressed protein	-1.02	$3.21x\ 10^{-06}$	Unclassified transcribed
7	4715	2381	Methyl-CpG-DNA binding protein	-0.98	$2.18x\ 10^{-08}$	Nucleotide biosynthesis
8	4543	2340	Peroxidase POA1	-0.98	$1.63x10^{-09}$	Secondary metabolism
9	1967	3133	UDP-glucoronosyl	-0.95	$4.7x10^{-05}$	Transporter
10	6669	6438	Unnamed protein	-0.94	$1.6x10^{-04}$	Unknown
11	3292	777	Importin alpha-like protein	-0.92	$1.1x10^{-04}$	Transporter
12	4673	997	α-1,4 glucan phosphorylase	-0.92	$1.1x10^{-04}$	A.A & protein metabolism
13	4248	2293	Oxysterol-binding protein	-0.92	$3.74x10^{-08}$	Lipid metabolism
14	8427	2848	Geranyl geranyl transferase	-0.92	$2.58x10^{-06}$	Signaling & gene regulation
15	3028	3252	TolB protein	-0.90	$1.2x10^{-03}$	Unclassified transcribed
16	5412	3623	Ras-related protein RAB8-5	-0.86	$1.2x\ 10^{-05}$	Transporter
17	5172	3756	60S ribosomal protein L12	-0.85	$9.88x10^{-06}$	A.A & protein metabolism
18	4539	9889	Cdc2MsC like protein kinase	-0.85	$9.15x10^{-07}$	A.A & protein metabolism
19	8475	350	Uroporphyrin-III C-methyltransferase	-0.84	0.025	Other biosynthesis
20	7333	132	ATSWI3C	-0.82	$2.5x10^{-04}$	Signaling & gene regulation
21	7561	9015	Chorophyll binding protein	-0.82	$4.31\ x10^{-04}$	Primary energy metabolism
22	172	1883	Peroxidase precursor	-0.81	$3.92x10^{-08}$	Oxidoreductases
23	6053	427	Fibre protein Fb19 (*Gossypium sp.*)	-0.79	$9.96x10^{-06}$	Cell wall development
24	1030	1488	Auxin-repressed protein	-0.74	$5.44x10^{-05}$	Unclassified transcribed
25	269	2256	Lipid transfer protein 4	-0.70	$1.52x\ 10^{-07}$	Lipid metabolism

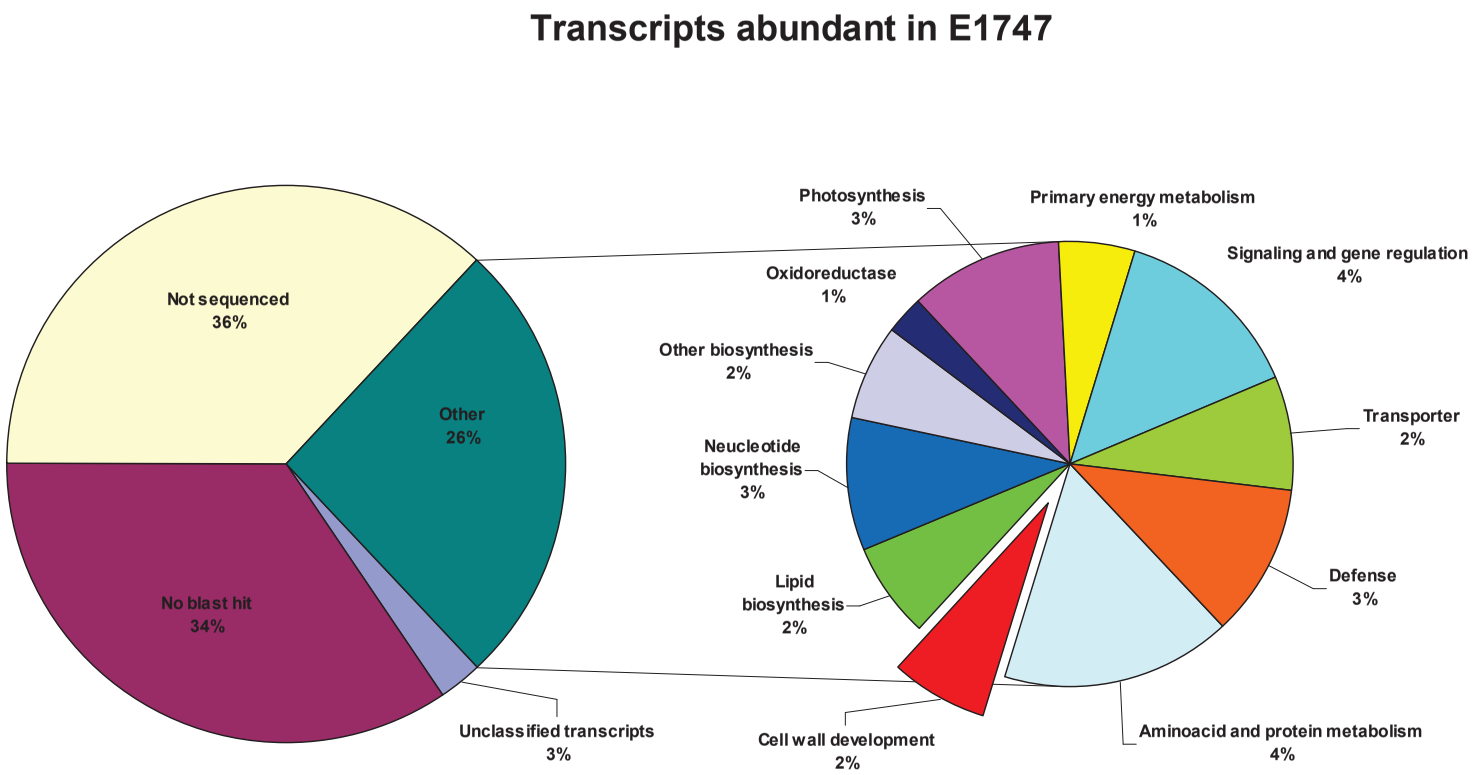


Figure 6.10. Functional classification of all unique probe sets/ transcripts found significant using significance analysis of microarrays (SAM) and more abundant in E1747 relative to Viking based on fold enrichment < 0.00 (log_2 signal intensity ratios - negative) with a false discovery rate of ≤ 5 %.

Table 6.3. Transcript abundance of selected probe sets in microarray comparisons of stem peels from Viking and E1747 along with their functional categories.

Functional Category	Probe set	# of Clones	BLASTx annotation in NCBI database	Fold enrichment (Viking/ E1747)
	7926	1	Callose synthase	1.95
Cell wall development	6100	1	Expansin	1.93
	1556	1	Ethylene-binding protein	1.75
	7120	1	HyPRP1 (*Gossypium sp.*)	1.08
	9	1	Beta-galactosidase	1.03
	224	1	Fasciclin-like AGP	1.01
	89	1	Galactose-binding protein	1.00
	5881	1	Chitinase-like protein (*Gossypium sp.*)	0.87
	4817	2	Beta glucosidase (*Hevea sp.*)	0.83
	152	2	Fasciclin-like AGP	0.79
	1555	1	Endo-1,4-beta-glucanase (*Gossypium sp.*)	0.77
	3726	1	Endo-1,4-beta-glucanase (*Gossypium sp.*)	0.73
	3301	1	Fibre protein Fb34(*Gossypium sp.*)	0.68
	143	1	Fructokinase	0.64
	239	1	Alpha tubulin	0.63
	4738	1	Pear beta-galactosidase	0.61
	8745	1	Fructokinase	0.60
	39	1	Alpha tubulin	0.59
	2820	1	Sucrose synthase	0.44
	82	2	Class IV chitinase	0.36
	26	8	Chitinase-like protein	0.32
	3359	2	CCR protein	-0.59
	4970	1	Galactose mutarotase	-0.60
	983	1	Galacturonosyl-transferase	-0.63
	6387	1	HyPRP1(*Gossypium sp.*)	-0.67
	6053	2	Fibre protein Fb19 (*Gossypium sp.*)	-0.79
Lipid biosynthesis	5171	1	GSDL-motif lipase	1.10
	374	1	ATP-citrate lyase	1.07

Functional Category	Probe set	# of Clones	BLASTx annotation in NCBI database	Fold enrichment (Viking/ E1747)
	183	1	Pyrophosphatase	0.17
	3674	1	Alpha-1,2-fucosyltransferase	-0.20
	2411	1	Delta-1,2-desaturase	-0.51
	800	13	Lipid transfer protein	-0.58
	269	28	Lipid transfer protein	-0.70
	4248	1	Oxysterol-binding protein	-0.92
Oxidoreductase	665	1	N-hydroxylating cytochrome P450	1.74
	158	3	N-hydroxylating cytochrome P450	1.40
	4341	1	Acetohydroxy isomeroreductase	1.20
	7685	1	Ketol-acid reductoisomerase	1.19
	120	1	Protochlorophyllide oxidoreductase	1.16
	1886	1	N-hydroxylating cytochrome P450	0.98
	512	1	Peroxidase	0.79
	2038	1	Cytochrome b5 reductase	0.78
	434	1	Blue Copper Protein	0.63
	7307	1	N-hydroxylating cytochrome P450	0.60
	1211	2	Cytochrome P450 protein	0.59
	305	1	Cytochrome P450 protein	0.58
	1250	1	6-phosphogluconate dehydrogenase	0.34
	925	1	Monooxygenase	-0.41
	172	8	Peroxidase precursor	-0.81
Transporter	4010	1	MipC	1.95
	5216	1	ATP-binding protein	0.90
	591	1	ATP synthase	0.69
	225	4	Aquaporin	0.65
	111	1	Aquaporin	0.59
	236	1	Aquaporin	0.38
	214	1	Delta-tonoplast intrinsic protein	0.29
	1125	1	C2 domain-containing protein	-0.41
	210	1	16kDa Membrane protein	-0.47

Functional Category	Probe set	# of Clones	BLASTx annotation in NCBI database	Fold enrichment (Viking/ E1747)
	6186	1	Mannitol transporter	-0.69
	5412	1	Ras-related protein RAB8-5	-0.86
	3292	1	Importin alpha-like protein	-0.92
	1967	1	UDP- glucoronosyl	-0.95
Unclassified transcribe	656	1	Hypothetical protein	0.93
	7736	1	Hypothetical protein	0.87
	8662	1	Hypothetical protein	0.66
	756	1	Hypothetical protein	0.65
	3936	1	Hypothetical protein	0.60
	70	1	Hypothetical protein	-0.24
	8371	1	Hypothetical protein	-0.42
	7485	1	Hypothetical protein	-0.52
	7884	1	Hypothetical protein	-0.61
	1030	2	Auxin-repressed protein	-0.74
	3028	1	TolB protein	-0.90
	99	8	Auxin-repressed protein	-1.02

Quantitative real -time PCR validation

To confirm that RNA expression patterns identified by microarray analysis were reproducible using other techniques for measuring transcript abundance, six transcripts were selected as targets for quantitative real- time PCR. These transcripts were selected to represent a variety of functional categories and expression patterns so that the results might be used to validate the microarray data in general.

The highest transcript abundance was found in probe set # 158 with high sequence similarity to N-hydroxylating cytochrome P450, and a fold enrichment of ≥ 5.8 in Viking relative to E1747. The transcript that was found highly abundant in E1747 as compared to Viking encoded for lipid transfer protein (probe set # 269) with a fold enrichment of ≥ 1.9. A table comparing the transcript abundance detected by both microarray and quantitative real- time PCR (qRT-PCR) is depicted in Table 6.4.

In Figure 6.11, the relative expression of these target genes are depicted in a graph using the data from both qRT-PCR and microarrays provided in Table 6.4. The overall expression patterns were qualitatively consistent between both the analyses. However, there were differences in the level of quantification, which may be due to different sensitivities of the detection systems.

Table 6.4. Comparison of log_2 signal intensity ratios (Viking/ E1747) obtained by qRT-PCR and microarray analysis.

	Probe set	UID	BLASTx annotation in NCBI database	Microarray		qRT-PCR	
				log_2 ratio	(±) SE	log_2 ratio	(±) SE
1	4654	996	Ubiquitin conjugating enzyme-like	-1.333	0.030	-0.728	0.154
2	172	324	Peroxidase precursor	-0.809	0.014	-1.606	0.049
3	269	156	Lipid transfer protein 4 precursor	-0.696	0.005	-1.942	0.133
4	152	1833	Fasciclin-like AGP 4	0.789	0.029	0.282	0.166
5	9	4561	beta-galactosidase	1.034	0.020	0.512	0.101
6	158	6071	N-hydroxylating cytochrome P450	1.403	0.204	5.869	0.556

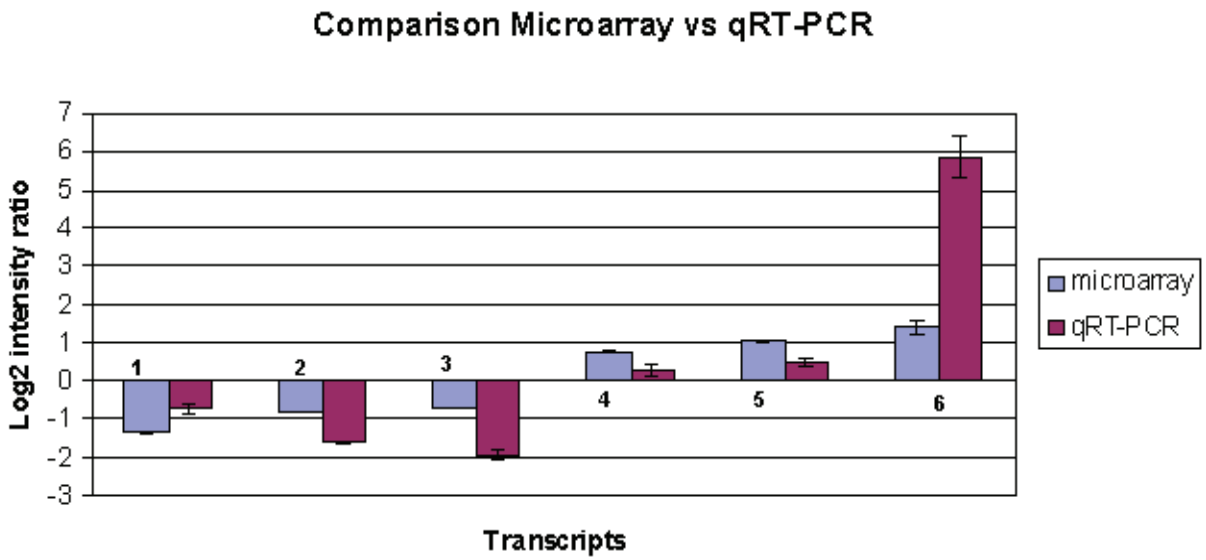


Figure 6.11. Graphical representation of log_2 ratios obtained by qRT-PCR and microarray analysis for the six transcripts mentioned in Table 6.4. Data shown are means of three biological independent replicates, error bars represent standard errors.

Discussion

At present there has very little transcriptome profiling of bast fibre tissues to identify the differences between fibre and oilseed flax types (Fenart et al., 2010). The present study indicates a number of genes differentially expressed in the fibre rich phloem tissues of both the flax types. This may suggest targets to improve the fibre characteristics with respect to both quantity and quality in Canadian oilseed flax varieties.

The fibre concentration in the fibre flax cultivar, Viking was 19.64 %, whereas the fibre concentration of oilseed flax genotype, E1747 was 15.64 % as revealed in Chapter 3 (Table 3.6)**.** The significant differences in flax stem anatomy and fibre cell characteristics as revealed by anatomical study (Chapter 4, Figure 4.3) indicated that these two diverse flax genotypes were good candidates for the microarray study. Interestingly, the stem cross-sectional anatomy of these two genotypes (Figure 6.1) revealed that both Viking and E1747 start producing fibre cells as early as two to three weeks after germination. The key differences between the two flax types lie in the quantity and quality of fibre being produced. The potential transcripts identified in this microarray study could help to explain those differences.

Transcripts highly expressed in fibre type flax genotype (Viking)

The microarray comparison between Viking and E1747 identified several transcripts that fell under the broad functional category of cell wall development (7 %) with a greater abundance in Viking relative to E1747. Some of the most abundant and interesting transcripts of this functional group are discussed below.

Callose is a polysaccharide found in the cell walls of a variety of higher plants. Callose plays important roles during a variety of processes in plant development and/ or in response to multiple biotic and abiotic stresses (Chen and Kim, 2009). A high abundance of callose synthase with a fold enrichment of ≥ 1.94 was detected in Viking relative to E1747 (Tables 6.1 and 6.3). This is interesting as the deposition of callose at the cell plates precedes the synthesis of cellulose (Kakimoto and Shibaoka, 1992; Samuels et al., 1995), which is a major component of fibre cells.

Callose synthase is localized mostly in growing cell plates, and may form a complex with UDP-glucose transferase to facilitate the transfer of substrate for callose synthesis (Stone and

Clark, 1992). In relation to plant fibres, callose deposition and degradation was found correlated with the closure and opening of cotton fibre plasmodesmata (Ruan et al., 2004). Using confocal imaging, the duration of the plasmodesmata closure was found positively correlated with fibre length in three tetraploid and two diploid cotton genotypes. Flax fibre cells undergoing coordinated growth during early stages of development have plasmodesmata in their cell walls. However, at the onset of intrusive growth and during subsequent fibre development, plasmodesmata were found to be disrupted (Ageeva et al., 2005; Snegireva et al., 2010). This indicates that callose synthases in the plamodesmata may have a prominent role in fibre cell elongation and development.

In this study, Viking phloem tissues were found rich in expansins with ≥ 1.93 fold enrichment relative to E1747. In various cell types expansins are the primary agents inducing cell wall expansion. It is believed that they disrupt hydrogen bonds between cellulose and hemicellulose, thereby allowing cell wall polymers to yield the turgor-generated growth force (Carpita and McCann, 2000; Snegireva et al., 2010). Achievement of relatively slow elongation without the closure of plasmodesmata may be accompanied through the relaxation of cell wall mediated by the expansin genes (Ruan et al., 2000). High levels of expression of an expansin gene have also been observed in cotton fibres early in elongation (Shimizu et al., 1997; Orford and Timmis, 1998; Ruan et al., 2001) when the plasmodesmata were open.

Phytohormones regulate numerous aspects of plant growth and development. Various studies on cotton fibre cell development have also implicated plant hormones as critical regulators of fibre development and differentiation (Kim and Triplett, 2001). The plant hormone ethylene participates in the regulation of a numerous developmental processes and serves as a key mediator of plant responses to biotic and abiotic stress factors (Stepanova et al., 2005). Studies indicate that ethylene plays a major role in promoting cotton fibre elongation by increasing the expression of sucrose synthase, tubulin**,** and expansin genes (Riechmann and Meyerowitz, 1998; Shi et al., 2006). Interestingly, the present study also detected an ethylene binding protein with ≥ 1.75 fold enrichment in Viking relative to E1747 fibre rich tissues.

Other cell wall related transcripts that were abundant in the fibre rich tissues of the fibre flax genotype Viking (≥ 1.30 fold enrichment) relative to E1747 included arabinogalactan proteins (AGP) and fasciclin-like AGPs (FLAs). AGPs have been implicated in diverse

developmental roles such as differentiation, cell-cell elongation, somatic embryogenesis and programmed cell death (Showalter, 2001) but their exact functions remain unclear. FLAs have been described to be highly expressed in the mid segment of flax stem peels (Roach and Deyholos, 2007) indicating that the FLAs may be specifically involved in the transition of phloem fibres from elongation to secondary cell wall thickening. FLA genes were also highly expressed in poplar tension wood where they have been hypothesized to be involved in the formation of cellulose –rich gelatinous fibres (Lafarguette et al., 2004; Mellerowicz and Sundberg, 2008; Fenart 2010).

In addition, β-galactosidase and galactose-binding like proteins were also detected in Viking (≥ 0.83 fold enrichment) relative to E1747. β -galactosidases cleave galactose subunits or other galactose containing cell wall polymers and are reported to be involved in regulating cell wall porosity and rigidity (Martin et al., 2005; Minic and Jouanin 2006). β-glactosidase expression was significantly higher in the snap point region of flax phloem tissues indicating its role in cell wall remodeling and transition (Roach and Deyholos, 2007 and Fenart et al., 2010). In a recent study, developing stems of transgenic (LuBGAL- RNAi) flax with reduced β – galactosidase activity showed significant reduction in aggregation and crystallinity of cellulose microfibrils in flax stems (Roach et al., 2011) confirming the importance of β-galactosidase in fibre development and remodelling.

Other functional group transcripts highly abundant in Viking included transporters such as major intrinsic proteins (MipC) with ≥ 1.95 fold enrichment. Mips potentially encode aquaporins that regulate water transport in most plants (Yamada et al*.,* 1995). Two other aquaporins (probe set # 225 and 236) were detected in abundance in Viking, supporting previous studies that water transporters can influence the growth and expansion of cell wall through turgor pressure (Liu et al., 2008). In addition, mitogen-activated protein kinases (MAPKs), which are usually responsible for cell wall loosening during the rapid fibre elongation period in cotton (Ji et al., 2003) were also more abundant in Viking (≥ 1.49 fold increase in Viking) relative to E1747.

Another highly abundant transcript was N-hydroxylating cytochrome P450 which had ≥ 1.74 fold enrichment in Viking relative to E1747. The cytochrome P450 falls under the functional group of oxidoreductases and is potentially involved in a range of diverse processes such as of lignin biosynthesis, hormones (gibberellins, brassinosteroides, auxin) and defense

(isoflavonoids, hydroxamic acid, glucosynolates, cyanogenic glucosides and terpenes) (Dasgupta et al., 2011). With regard to plant hormones, cytochrome P450 monooxygenases are responsible for regulating several steps of gibberellic acid biosynthesis. Cytochrome P450 monooxygenases are also involved in the biosynthesis of brassonosteroids, a class of another plant hormone that has been shown to affect stem elongation and vascular tissues development (Rademacher, 2000; Clouse and Sasse, 2003). In a recent study by McKenzie and Deyholos (2011), exogenous application of Paclobutrazol (PBZ) a Cytochrome P450 inhibitor involved in gibberellic acid biosynthesis showed a reduction in the number of fibre cells, fibre cell length and overall reduction in plant height in flax. In another study using microarrays a significant abundance of cytochrome P450 was found during early cotton fibre elongation (Ji et al., 2003) confirming the importance of cytochromes in fibre growth and development.

Surprisingly, no cellulose synthase transcripts were detected in the microarray experiment even though cellulose is a major constituent of flax fibres. This may be due to both flax types producing the cellulose at a comparatively similar rate at the seedling stage, and only during the later part of secondary cell wall development of fibre cells, that cellulose deposition is distinctively different between the two flax types. However, further studies are needed to strengthen this hypothesis.

Transcripts highly expressed in oilseed flax (E1747)

Most of the transcripts abundant in E1747 fell under the functional group of amino acid and protein regulation; and signaling and gene regulation. The highest abundant transcript in E1747 did not align with any known gene. The next abundant transcript were ubiquitin conjugating enzymes ($\geq$ 1.33 fold increase in E1747), which usually performs the second step in the ubiquitination reaction and targets a protein for degradation via the proteasomes (Nandi et al., 2006). Ubiquitin- mediated protein degradation pathways are highly upregulated during the secondary cell wall thickening in cotton fibre cells (Ghazi et al., 2009). Albady and Wikins (2007) also noted the importance of ubiquitin protein degradation during secondary cell wall thickening of Pima cotton varieties and suggested that specific ubiquitin ligase alleles may even be associated with fibre quality quantitative trait loci (QTLs). The detection of more ubiquitin conjugating enzyme transcripts could be one of the reasons for observing smaller fibre cells and

poor fibre concentration in E1747 as compared to Viking (Chapter 4). However, further research would be required to confirm this hypothesis.

As previously discussed, cellulose is the major component of flax fibre cell walls. UDP-glucose is the major substrate for the synthesis of cellulose (Saxena and Brown, 2005). UDP-glucose glucosyl transferase is one of the sugar transporters that may divert UDP-glucose from cellulose synthase enzymes and builds short glucosides such as calloses (Hong et al., 2001). In a study by Roach and Deyholos, (2008) UDP-glucose glucosyl transferase was found highly enriched in 7-d elongation stage of flax hypocotyl. In the present study, UDP- glucose glucosyl transferase family protein (probe set # 1967) was highly abundant in E1747 (≥ 0.95 fold increase) relative to Viking (Table 6.2) indicating the possible role of these enzymes in cell wall deposition.

Plant hormones as previously mentioned are key regulators in growth and development of plant cells. Auxins in particular, is known to be involved in many plant developmental processes including acid induced cell wall loosening and vascular development (Taguchi et al., 1999; Fukuda, 2004). While many genes up-regulated by auxin have been characterized, comparatively less is known about the genes that are down – regulated. Auxin repressed protein (ARP) in this study had ≥ 1 fold enrichment in the E1747 as compared to Viking (Table 6.2). A study by Wang et al. (2007), indicated that the presence of ARPs might contribute to the retardation in longitudinal growth of the mutant type fir. Another study on a transgenic tree legume (*Robinia pseudoacacia*) found that the *RpARP* gene is post transcriptionally regulated and negatively associated with shoot elongation (Park and Han, 2003). Interestingly, E1747 and other oilseed cultivars are shorter in height in comparison to fibre flax cultivars including Viking (Chapter 3), suggesting the possible role of ARPs in longitudinal plant growth.

Another important transcript family are the peroxidases (≥ 0.98 fold enrichment in the E1747 relative to Viking) that are believed to be responsible for the final condensation of cinamyl alcohols to form the lignin monomer. Lignin biosynthesis is closely related to secondary cell wall deposition and thickening of vascular cell walls imparting strength and rigidity to the plant structure (Minorsky, 2002, 2003). However, lignin and peroxidases have often been implicated in degrading quality in jute fibres and are used as biochemical markers for selecting varieties with improved fibre quality (Sengupta and Palit, 2004; Sinha et al., 2008). With relation

to flax, further biochemical analysis of the flax fibres for lignin and peroxidase estimation may be needed to clearly understand the biochemical differences in fibre quality between the two flax types.

Lipid transfer proteins (LTP) were also found more abundant in the oilseed genotype with ≥ 0.71 fold enrichment relative to fibre flax genotype. LTPs are thought to be involved in the trafficking of fatty acids and enhance the *in vitro* transfer of phospholipids between membranes binding to acyl chains. On the basis of these properties, LTPs were thought to participate in membrane biogenesis and regulation of the intracellular fatty acid pools (Kader, 1996). Many of the cotton genes encoding nonspecific lipid transfer proteins and enzymes are found to be involved in various steps during fatty acid chain elongation and highly upregulated during early fiber development (Ji et al., 2003; Qin et al., 2005, 2007b; Shi et al., 2006; Gou et al., 2007). In flax, a high abundance of LTP transcripts were also found to correlate with different stem development stages of flax seedling (Roach and Deyholos, 2007). Putative ESTs encoding LTPs were also found enriched in the fibre peels of flax cDNA library (Day et al., 2005b). These reports suggest that the biosynthesis of very-long-chain fatty acids (VLCFAs) or their transport may be essential both for seed oil biosynthesis as well as for fibre development processes (Fenart et al., 2010).

In conclusion, this study has helped to identify several candidate genes for phloem fibre specific processes that can help us in understanding the mechanisms involved in fibre development in the flax stem. The quantitative real time PCR experiment validated the over all accuracy of the microarray platform (Table 6.3, Figure 6.11). However, it is important to stress that the mRNA and protein levels do not always correlate, so further functional characterization of the gene products is warranted. In addition these results provide a snapshot of the transcript abundance in a particular sample at a particular time. Nevertheless, understanding the molecular basis of stem fibre biosynthesis is of critical importance in designing strategies for improving fibre quantity as well as quality. Identification of these candidate genes will further tailor the application of both classical and molecular breeding towards expanding the knowledge on fibre biosynthesis in flax and other bast crops.

CHAPTER 7

General Discussion and Conclusions

General discussion

Flax is often considered a total utilization crop because of the potential to extract value from two distinct products - seeds and stem fibres. Canada leads the world in oilseed flax exports and produces almost 40 % of global production. Saskatchewan produces more than 70 % of the total flax grown in Canada (SaskFlax, 2011) and had an export value of $254.6 million in 2010 (Saskatchewan Ministry of Agriculture, Agricultural Statistics, July 2011). However, flax continues to lose acres to other oilseed crops such as canola due to its lower yield potential. In order for Canada to retain its competitive edge as the world's leading exporter of flaxseed, crop diversification avenues through stem fibre utilization is thought to be important.

This research provides several new insights into flax fibre genetics. Evidence provided in Chapter 3 confirms that fibre content is a multi-genic trait and follows the quantitative inheritance mode (Keijzer and Metz, 1992). For the first time in Canada a fibre × oilseed RIL population was evaluated for both fibre and oil related traits. The economically important fibre and seed characteristic that were tested in this study showed great consistency over multiple locations. The study showed significant variability for stem fibre content in the Viking × E1747 RIL population indicating the possibility of further improving this trait. The study also identified several transgressive segregants in the RIL population for an array of traits including fibre concentration, protein concentration, linoleic and linolenic acid concentration for use in further breeding program. However, the presence of a significant G × E interaction for fibre concentration would make selection for this trait challenging (Dimmock et al., 2005).

An appreciation of anatomy is fundamental to an understanding of many aspects of plant biology, including the phenotypic and molecular ends of the spectrum. Flax stem anatomy and ultrastructure of fibre bearing tissues has been earlier studied due to their wide use in the textile industries (Esau, 1943; Shepherd, 1956; Himmelbach and Akin, 1997). However, comparative

differences in flax stem anatomy with respect to the two flax types, have been very limited (Sankari, 2000a). In Chapter 4, the study establishes an anatomical basis for further research into flax stem anatomy with special reference to the differences between fibre flax and oilseed flax stems. The study also looked into the possibility of screening high fibre flax lines based on various anatomical region measurements using the Viking × E1747 RIL population as previously described in Chapter 3.

Why use an anatomical study under controlled conditions when field study using NIR fibre estimation would do?

The results from Chapter 4 supports the use of anatomical study for quick screening of high fibre genotypes under controlled conditions such as a growth chamber. The anatomical study was able to pick up significant differences between high fibre RILs vs. low fibre RILs using as little as three cross-sections made from three individual plants grown in pots under growth chamber conditions. In contrast, the NIR requires at least a sample size of a hundred mature flax plants in order to make valid fibre concentration measurements as mentioned in Chapter 3 (Burton, 2007).

Among the three different growth stages evaluated in this study namely; seedling, green capsule and physiological maturity, the green capsule stage has been identified as the earliest stage where effective screening of the high fibre genotypes can be performed using growth chamber plants. Previous studies have also indicated the importance of this growth stage during harvesting for linen production in flax (Barber, 1991; Deyholos, 2006). Studies in other bast fibre crops such as Jute (*Corchorus capsularis*) have emphasized the importance harvesting during early pod development stage for the production of maximum fibre yield and fibre strength (Shamsuzzaman et al., 1998). In addition, several researchers have conducted both histological and molecular studies specifically at the green capsule stage indicating the significance of this stage for fibre related research in flax (Gorskova et al., 2003; His et al., 2001; Fenart et al., 2010). Anatomical evaluation at the green capsule stage could save a considerable amount of time for the flax breeders as the field evaluation using NIR requires plants to be grown up to seed maturity, harvested and then cut into 19 cm long straw bundles in order to be analyzed by the NIR instrument.

The anatomical study presented here was used to evaluate an array of different anatomical regions to identify differences in stem anatomy of the diverse flax lines. Based on the results, three anatomical markers/ traits, namely average area of single fibre cells, total fibre area and fibre to stem area ratio, were found to be important. These three anatomical markers were sufficient to accurately differentiate between high and low fibre containing flax genotypes. The above mentioned anatomical markers were also strongly correlated ($p < 0.01$) with the NIR fibre estimation data (Table 4.5.3) confirming the importance of the above mentioned anatomical markers for screening high fibre genotypes. Consequently, this observation also validated that the NIR instrument was actually measuring only the fibre related regions in the stem and not other vascular regions such as epidermis, cortex or xylem.

Is anatomical screening as fast as NIR screening?

The anatomical measurements can be easily performed by hand made flax stem cross-sections. An optical microscope fitted with a digital camera and digital imaging software is sufficient to make all the anatomical measurements. The level of technical expertise required for the anatomical screening is comparable to that required for NIR fibre estimation. However, the time required for making the cross-sections and completing digital measurements per sample can is more time consuming relative to the NIR fibre estimation.

What would anatomical screening miss?

The anatomical data from the growth chamber plants would fail to capture a part of environmental variability that can be only found under field conditions due to factors such as such as, precipitation, soil type and growth temperatures. However, the above mentioned anatomical markers, especially average area of single fibre cells and total fibre area, had significant correlations ($p < 0.01$) between the Kernen Farm and growth chamber studies (Table 4.6), indicating that irrespective of where the plants grew, anatomical screening could assist in the screening of high fibre genotypes.

Improvement of flax with regard to fibre concentration can also be assisted through the development of molecular resources. Molecular markers provide valuable data on population diversity through their ability to detect variation at the DNA level (Fu et al., 2002; Adugna et al., 2005; Domyati et al., 2011). In Chapter 5, the molecular diversity within the Viking × E1747

RIL population and the two flax parents were investigated by means of SSR and CAPS molecular markers. Genetic similarity of only 37 % between the parents indicated high variability between the parents at the genomic level and that Viking × E1747 RIL population can be used for identifying genes/ markers linked with fibre as well as oil related traits (Klocke, 2000; Vroman, 2006).

Though the study used a limited number of molecular markers, there were significant marker trait associations (using field data from Chapter 3) for traits such as plant height, oil and fatty acid concentrations ($p < 0.01$). These marker trait associations were tested using both single marker analysis as well as step wise regression analysis indicating their reproducibility in diverse environments (Chandra et al., 2004).

The identification of these easy to use, highly reproducible polymorphic markers would directly be applicable to larger flax mapping projects such as the Total Utilization of Flax Genomics (TUFGEN) led by Dr. Gordon Rowland and Dr. Sylvie Cloutier, which aims at providing genetic knowledge for the improvement of seed and fibre traits of oilseed flax.

In Chapter 6, global transcript profiling using cDNA - based microarrays was performed to identify differentially expressed fibre related transcripts between the two diverse flax types, Viking (fibre flax) and E1747 (oilseed flax). The study identified several transcripts with specific cell wall functions that were highly abundant in Viking relative to E1747. For example, the study picked up transcripts such as callose synthase (Ruan et al., 2004), expansin (Carpita and McCann, 2000), cytochrome P450 (Rademacher, 2000; McKenzie and Deyholos, 2011), AGP (Roach and Deyholos, 2008; Fenart et al., 2010) and GAL (Roach et al., 2011), which were highly abundant in the fibre genotype relative to the oilseed genotype.

The study also picked up interesting transcripts highly abundant in E1747 relative to Viking such as auxin repressed proteins (Wang et al., 2007), peroxidases (Sengupta and Palit 2004; Sinha et al., 2008) and lipid transfer proteins (Ji et al., 2003; Gou et al., 2007), indicating their role in oilseed fibre development. As mentioned earlier, the study also identified several phytohormones related transcripts indicating their significance in fibre growth and development (Gialvalis and Seagull, 2001; McKenzie and Deyholos, 2011).

With the recent development of the CDC Bethune genome assembly under the previously mentioned TUFGEN initiative, these candidate genes could be of great value for future molecular studies and to increase the number of molecular resources available towards fibre improvement in flax.

Is it possible to breed dual purpose flax in western Canada?

There are several limitations for breeding new dual purpose cultivars to meet both the markets of oil and fibre. Preliminary studies by Burton (2007) showed fluctuating amounts of fibre between cultivars and it was unclear, which oilseed cultivars could consistently yield high fibre concentration under western Canadian growing conditions. A consistent high fibre concentration of the flax straw is required for fibre from oilseed flax to be economically viable. The study presented here identified five RILs (341, 264, 350, 337, 306) which had consistently high fibre concentration when grown under multiple locations (Chapter 3, Table 3.7.1).

Opinions vary on the best way to approach the breeding of dual purpose flax as previously mentioned in Chapter 2. The present study used one of the approaches recommended by Foster et al. (1997) and Vroman (2006) by evaluating a RIL population derived from crossing an oilseed x fibre flax as parents for selecting potential dual purpose lines.

A significant genetic variability within the Viking × E1747 RIL population with respect to both fibre as well as oil related traits indicated the possibility of improving both fibre and oil concentration in flax (Chapter 3, Table 3.5). It also suggests that breeders could choose genotypes on the basis of fibre or oil or both traits and thereby respond effectively to different market needs. A lack of tight association between fibre and oil related traits were indicative that both the traits could be simultaneously improved in a single cultivar (Table 3.9). Studies conducted by Kaul et al. (1994) and; Diederichsen and Ulrich (2009) also indicated a lack of significant correlation between fibre and oilseed characteristics in the field trials strengthening the hypothesis that breeding for dual purpose flax types is possible in western Canada.

The results also indicated a lack of strong association between plant height and fibre concentration (Chapter 3) and between plant height and fibre related anatomical traits (Chapter 4) suggesting that fibre concentration could be improved in the short Canadian oilseed varieties. Results from both these studies as well as previous research indicated that plant height were

strongly associated with xylem area in the stems as compared to any other anatomical regions of the stem (Ye, 2002; Pederson et al., 2005; Banniza et al., 2005). A recent study (McKenzie and Deyholos, 2011) using plant growth regulator treatments in flax also indicated that plant height had significant association with stem diameter and xylem radius confirming the above mentioned hypothesis.

In the current study (Table 3.6), the absolute difference among mean values for fibre concentration in fibre flax (19.76 %) and oilseed flax cultivars (16.10 %) were usually low (3.6 %). However, in relative terms this difference is a large difference (18.21 %). Similarly, the difference in fibre concentration between oilseed flax cultivars (E1747, Flanders, CDC Somme- mean 16.10 %) and the five potential dual purpose flax lines selected (RIL319, 311, 273, 309, 287- mean 18.92 %), indicated that in relative terms the potential dual purpose lines exceeded oilseed cultivars by 14.90 % in stem fibre content (Table 3.8.1). Such a difference may greatly influence fibre yield, even if fibre is only a bi-product from a variety grown mainly for its seed oil.

However, a significant G x E interaction coupled with a moderate heritability for fibre concentration (Chapter 3) indicates that further field testing over several locations and years would be required to make effective selections. The anatomical and molecular resources generated in Chapters 4, 5 and 6 of this thesis would help in developing breeding tools for assisting in dual purpose variety selections in the near future.

It is unlikely that the dual purpose varieties would be able to match the European fibre flax varieties in terms of fibre yield and fibre quality due to environmental constraints such as short growing seasons and cooler temperatures of the prairies. In addition, some degree of compensation may also be expected between the seed and stem fibre components due to physiological reasons (Dimmock et al., 2005). However, the results from this study indicate that it is possible to improve the fibre concentration trait in the current oilseed cultivars and bring them at par with the fibre flax cultivars to make flax production in Canada viable for both oil and fibre industries.

Conclusions

1. There is significant genetic variability within the Viking × E1747 RIL population with respect to both fibre as well as oil related traits.
2. There is significant G × E interaction controlling fibre inheritance. However, there is absence of any cross over interactions. Fibre concentration in this study was moderately heritable ($h^2_{b.s}$).
3. Lack of significant negative correlation between fibre traits and oil related traits indicates that it is possible to breed for dual purpose flax varieties with improved stem fibre and seed oil.
4. Significant differences observed between the stem anatomical features of fibre flax and oilseed flax genotypes indicate the possibility of improving fibre concentration based on anatomical traits.
5. It is possible to select high fibre flax genotypes based on anatomical markers such as average area of single fibre cells, total fibre area and fibre to stem area ratio.
6. The study supports the use of controlled environments such as growth chambers for the purpose of early screening of high fibre flax genotypes at the green capsule stage using above mentioned anatomical markers.
7. Correlation studies have confirmed that anatomical methods are as useful as NIR fibre estimation thereby providing flax breeders with additional selection tool with respect to bast fibre improvement.
8. Genetic similarity of only 37 % between the parents based on 19 molecular markers confirmed that Viking × E1747 RIL population is a promising mapping population to identify genes / markers related to both fibre as well as oil related traits.
9. Microarray study has provided a high throughput platform for successfully identifying potential transcripts differentially expressed in the diverse flax types (Viking and E1747), indicating their importance in future flax fibre research.

Future Directions

This thesis describes the application of both conventional and latest molecular breeding methods for addressing fibre improvement in oilseed flax. At the same time the results are helpful in understanding the genetic basis of fibre concentration in greater details. Based on the results obtained in Chapter 3, future work should be directed towards:

1. Further phenotyping of the Viking × E1747 RIL population over years and locations should be done to improve the estimation of heritability and reduce the impact of G × E interaction on the measurement of traits, especially fibre concentration. Also widening the range of phenotypic traits under study to include fibre yield, seed yield, days to flowering, days to maturity and pest resistance would help in better understanding the agronomics and extent of adaptability of the potential flax lines under western Canadian growing conditions.

2. Due to the presence of a wide range of variability in the Viking × E1747 RIL population, further investigation to identify genotypes with diverse end uses including specialty oil selection such as high or low palmitic, stearic and oleic fatty acids could be rewarding (Appendix A, Table A.3).

The anatomical study in Chapter 4 took into account a wide range of anatomical traits measured under both controlled and field environments. However, there were certain areas that were out of the scope of the present study. Further studies using flax stem anatomy should be directed towards the following areas:

1. The present study undertook only cross-sectional analyses of flax stems. It would be interesting to study the longitudinal sections of flax stem especially fibre cell length and its development over different growth stages. Differences between fibre flax and oilseed flax based on the longitudinal sectioning of flax stems may add to our understanding of fibre development and improvement in current oilseed flax varieties.

2. There was some ambiguity between previous research (Sankari, 2000a) and the current study in determining the average area of single fibre cells or size of ultimate fibre cells between the fibre flax and oilseed flax types as discussed in Chapter 4. Further studies using different fibre and oilseed flax varieties should be conducted in order to make more definitive conclusions regarding differences between the average sizes of the fibre cells in the two diverse flax types.

3. Though anatomical study was successful in differentiating high (~20.00 %) vs. low fibre (~15.00 %) genotypes, it was not possible to clearly differentiate the intermediate types such as the potential dual purpose lines. Further research should be conducted to standardize selection criteria in order to make efficient selection for both high and the intermediate fibre genotypes.

4. Cortex width was the only anatomical trait found significantly different in the dual purpose lines as compared to the high / low fibre lines from green capsule stage onwards, indicating a possibility of using contex width as an anatomical marker for dual purpose selection (Table 4.4.1). However, further research in this direction is needed to substantiate this hypothesis.

Results from Chapter 5, indicate that molecular marker assisted selection could be of great potential in obtaining genotypes with improved fibre as well as oil related traits. Future work following from the molecular marker study should be directed towards:

1. Elaboration and integration of molecular markers from other SSRs discovered recently (Deng et al., 2010, Ragupathy et al., 2011; Cloutier et al., 2009) into the Viking × E1747 mapping population. The markers reported in this study could be used in conjuncture with the much larger TUFGEN mapping project to improve the flax genetic map. Increasing the marker density of the linkage map would improve its usefulness by increasing the likelihood that markers will be tightly linked to the genes of interest in future QTL mapping studies.

2. With the recent advance in flax mapping and genome sequence assembly using oilseed flax variety CDC Bethune, the physical locations of the markers with the potential marker trait association such as CV8824, 2D2, 5B6 and 8A11 might help to further characterize their functions with respect to fibre as well as oil improvement.

Results from Chapter 6 indicated that the microarray platform was able to accurately discriminate transcript profiles in flax stems between the two diverse flax types. However, as mRNA and protein levels do not always correlate, further functional characterization of the potential gene products is warranted. As the microarray study was conducted at the seedling stage using RNA extracted from the stem peels present below the snap point region of the flax stems, the results may not reflect transcripts expressed at the meristamatic or the snap point regions. Furture work following this study should be directed towards:

1. Further microarray hybridization experiments for comparing fibre vs. oilseed genotypes designed to take into consideration stem sections from top, middle and basal regions of the flax stem and other important growth stages such as green capsule stage.

2. In the present study the microarray chip was constructed using cDNA library developed from a popular oilseed flax variety Norlin. The results from the present study might miss detection of some transcripts that may be unique to fibre flax varieties. It would be worthwhile to generate a new cDNA library and develop gene chips derived from fibre varieties such as Hermes (Fenart et al., 2010) to perform microarray hybridizations.

3. It will be interesting to compare gene expression between oilseed vs. dual purpose and fibre vs. dual purpose flax lines to pin point genes that are unique to only dual purpose lines.

4. Development of single nucleotide polymorphism (SNP) molecular markers from the potential transcripts such as AGPs, β -galactosidases, LTPs and cytochrome P450 by comparing to full length sequences of the CDC Bethune genome.

In addition, with the recent advances in EST discovery under TUFGEN initiative, the numbers of annotated ESTs (~260,000) have been constantly growing (Venglat et al., 2011; Ragupathy et al., 2011). Also, with the CDC Bethune genome assembly under progress, there are several other avenues that could be pursued in order to elucidate the gene functions related to fibre improvement in flax. Chemical mutagenesis is another such avenue, which has a number of inheritant attractions mainly by bypassing GMO approaches and generating allelic series at any targeted locus, resulting in a change in function, reduced activity or specificity or knock out mutation (Henikoff and Comai, 2003). Since the first publication, Targeting Induced Local Lesions in Genomes (TILLING) has been a widely adopted strategy being used in several model organisms such as Arabidopsis and wheat (McCallum et al., 2000; Caldwell et al., 2004). With the recent advances in high throughput next generation sequencing technologies such as RNA-seq or "Whole Transcriptome Shotgun Sequencing" (WTSS) is also likely to be of immense potential to flax researchers in the near future.

There are still many gaps in our knowledge pertaining to fibre improvement in flax despite the recent significant progress. The large amount of data generated by the various “omic” approaches and the future direction suggested here would go a long way in providing valuable information for the flax breeders for improving fibre content in the existing oilseed flax varieties suitable for western Canada.

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Appendix A

Table A.1. Variance components along with their F-values for palmitic acid concentration, stearic acid concentration and oleic acid concentration in the 2006 multi-location field trial grown at three locations; Melfort, Floral and Kernen.

Parameters	df	Palmitic acid concentration (%)	Stearic acid concentration (%)	Oleic acid concentration (%)
Location	2	0.30	51.51**	28.89*
Genotype	94	18.33***	22.22***	12.67***
Genotype- Melfort	94	6.86***	17.71***	6.50***
Genotype- Floral	94	8.51***	14.35***	6.39***
Genotype- Kernen	94	5.72***	3.67***	3.31***
Genotype × Environment	188	1.03	1.18	1.04

*significance at p= 0.05, ** significance at p= 0.01, *** significance at p= 0.0001.

Table A.2. The mean phenotypic values of palmitic, stearic and oleic fatty acid concentration for the parents (Viking and E1747) and the checks (Hermes, Flanders and Somme) in the 2006 multi-location field trial grown at three locations; Melfort, Floral and Kernen.

Palmitic acid concentration (%)				
Parents & check	**location**			**Mean**
	Melfort	**Floral**	**Kernen**	
Viking	5.44	5.10	5.24	5.26
E1747	6.84	7.01	6.67	6.84*
Hermes	5.44	5.55	5.82	5.60
Flanders	5.44	5.71	5.42	5.52
Somme	5.87	5.88	5.85	5.86*

Stearic acid concentration (%)				
Parents & check	**location**			**Mean**
	Melfort	**Floral**	**Kernen**	
Viking	3.50	4.08	4.16	3.91
E1747	3.64	3.90	4.01	3.85
Hermes	3.68	3.97	3.87	3.84
Flanders	3.73	4.40	4.33	4.15*
Somme	3.08	3.11	3.48	3.22*

Oleic acid concentration (%)				
Parents & check	**Location**			**Mean**
	Melfort	**Floral**	**Kernen**	
Viking	18.21	18.14	19.86	18.74
E1747	14.57	14.40	14.82	14.59**
Hermes	18.90	19.51	20.30	19.57
Flanders	17.78	18.34	18.07	18.06
Somme	18.35	17.46	17.68	17.83

*, **, *** are significantly different from Viking, at 5 %, 1 % and 0.01 % probability respectively; pooled mean values showing no '*' do not differ significantly from Viking according to paired t-test.

Table A.3. The mean phenotypic values of five out of 95 flax RILs population with the highest and the lowest values for palmitic, stearic and oleic fatty acid concentration in the 2006 multi-location field trial grown at three locations; Melfort, Floral and Kernen.

Palmitic acid concentration (%)									
High					**Low**				
RIL	**Melfort**	**Floral**	**Kernen**		**RIL**	**Melfort**	**Floral**	**Kernen**	
297	7.44^{3}	7.66^{2}	7.66^{2}	7.59^{a}	**344**	5.33^{95}	5.46^{91}	5.63^{88}	5.47^{s}
260	7.44^{2}	7.76^{1}	7.46^{3}	7.55^{a}	**324**	5.68^{90}	5.55^{93}	5.43^{94}	5.55^{rs}
273	7.43^{4}	7.38^{3}	7.71^{1}	7.50^{a}	**255**	5.70^{88}	5.49^{94}	5.50^{93}	5.56^{q}
271	7.10^{7}	7.26^{4}	7.08^{5}	7.15^{b}	**312**	5.42^{94}	5.86^{88}	5.75^{84}	5.67^{pq}
322	7.21^{6}	7.17^{5}	6.84^{7}	7.07^{c}	**268**	5.65^{91}	5.73^{91}	5.68^{86}	5.68^{ps}
Stearic acid concentration (%)									
High	**location**			**Mean**	**Low**	**location**			**Mean**
RIL	**Melfort**	**Floral**	**Kernen**		**RIL**	**Melfort**	**Floral**	**Kernen**	
309	4.86^{1}	5.11^{1}	5.38^{1}	5.11^{a}	**264**	2.81^{94}	2.73^{95}	3.05^{94}	2.86^{s}
259	4.70^{3}	4.58^{6}	5.19^{2}	4.82^{b}	**335**	2.72^{95}	3.41^{79}	2.97^{95}	3.03^{rs}
326	4.39^{9}	4.63^{3}	5.07^{3}	4.70^{c}	**271**	2.91^{91}	2.98^{92}	3.43^{83}	3.11^{qr}
324	4.52^{5}	4.58^{7}	4.86^{7}	4.65^{d}	**273**	2.99^{88}	3.01^{91}	3.38^{87}	3.12^{qr}
344	4.49^{6}	4.50^{13}	4.84^{8}	4.61^{e}	**306**	3.01^{85}	3.14^{85}	3.31^{93}	3.15^{p}
Oleic acid concentration (%)									
High	**location**			**Mean**	**Low**	**location**			**Mean**
RIL	**Melfort**	**Floral**	**Kernen**		**RIL**	**Melfort**	**Floral**	**Kernen**	
270	18.96^{1}	18.54^{1}	19.24^{4}	18.91^{a}	**327**	13.19^{95}	13.66^{95}	13.41^{95}	13.42^{q}
264	18.88^{2}	16.92^{16}	19.27^{3}	18.36^{ab}	**271**	13.89^{91}	13.69^{94}	14.75^{92}	14.11^{pq}
279	18.04^{6}	17.91^{5}	19.00^{6}	18.31^{ab}	**305**	13.97^{89}	13.88^{93}	14.58^{94}	14.14^{pq}
277	18.17^{5}	17.12^{12}	19.40^{2}	18.23^{c}	**338**	13.27^{94}	14.63^{87}	15.21^{89}	14.37^{p}
268	17.55^{12}	17.33^{9}	19.78^{1}	18.22^{d}	**286**	13.94^{90}	14.10^{92}	15.13^{90}	14.39^{np}

(Values within a column followed by a same letter (s) do not differ significantly according to LSD multiple range test, $P<0.05$.; Values within a column followed by a number is the ranking of the RIL in that particular location)

Table A.4. Mean comparisons of potential dual purpose RILs in the 2006 multi-location field trial grown at three locations; Melfort, Floral and Kernen.

Palmitic acid concentration (%)					t- test	
Dual	**location**			**Mean**	**Viking (mean)**	**E1747 (mean)**
RIL	**Melfort**	**Floral**	**Kernen**		**5.24**	**6.84**
319	5.98^{73}	6.28^{56}	6.06^{58}	6.11^{zh}	*	**
311	6.08^{63}	6.60^{28}	6.29^{43}	6.32^{nz}	*	ns
273	7.43^{4}	7.38^{3}	7.71^{1}	7.50^{a}	**	ns
309	6.15^{58}	6.24^{58}	6.00^{64}	6.13^{wh}	*	**
287	5.97^{75}	6.06^{73}	6.01^{62}	6.01^{bl}	*	**
Stearic acid concentration (%)					**t- test**	
Dual	**location**			**Mean**	**Viking (mean)**	**E1747 (mean)**
RIL	**Melfort**	**Floral**	**Kernen**		**3.91**	**3.85**
319	4.08^{18}	3.98^{32}	4.82^{9}	4.29^{mn}	ns	ns
311	3.78^{40}	3.99^{31}	4.33^{26}	4.03^{wx}	ns	ns
273	2.99^{88}	3.01^{91}	3.38^{87}	3.12^{qr}	*	*
309	4.86^{1}	5.11^{1}	5.38^{1}	5.11^{a}	*	**
287	4.01^{22}	4.10^{25}	4.24^{34}	4.11^{st}	ns	*
Oleic acid concentration (%)					**t- test**	
Dual	**location**			**Mean**	**Viking (mean)**	**E1747 (mean)**
RIL	**Melfort**	**Floral**	**Kernen**		**18.74**	**14.59**
319	16.65^{28}	15.22^{72}	16.35^{60}	16.07^{qd}	*	ns
311	16.09^{42}	15.77^{53}	16.81^{44}	16.22^{nb}	**	**
273	14.50^{83}	14.79^{82}	15.50^{84}	14.93^{in}	**	ns
309	15.03^{72}	16.43^{27}	16.28^{62}	15.91^{ug}	*	ns
287	15.05^{69}	15.53^{64}	15.40^{85}	15.33^{ck}	*	ns

Values within a column followed by a same letter (s) do not differ significantly according to LSD multiple range test, $P < 0.05$.; Values within a column followed by a number is the ranking of the RIL in that particular location. *, **, *** , are significantly different from Viking and/ or E1747, at 5 %, 1 % and 0.01 % probability respectively; pooled mean values showing 'n.s' do not differ significantly from Viking and/ or E1747 according to paired t-test.

Table A.5. Pearson correlation coefficients of palmitic, stearic and oleic acid fatty acid concentrations with other fibre and oil related variables in flax for 2005 and 2006 field trials.

Phenotypic Traits		Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)
Fibre (%)	$r_{(M,F,K)}$	-0.01	-0.05	0.05
	r_k	-0.08	-0.10	0.14
Plant height (cm)	$r_{(M,F,K)}$	-0.14**	-0.15**	0.00
	r_k	-0.28*	-0.11	-0.05
Total oil (%)	$r_{(M,F,K)}$	0.10*	-0.11**	-0.03
	r_k	0.00	-0.10	0.07
Total protein (%)	$r_{(M,F,K)}$	-0.12**	0.12**	0.00
	r_k	-0.02	0.09	-0.16
Palmitic acid (%)	$r_{(M,F,K)}$		-0.08	-0.33***
	r_k		-0.07	0.14
Stearic acid (%)	$r_{(M,F,K)}$	-0.08		0.03
	r_k	-0.07		0.03
Oleic acid (%)	$r_{(M,F,K)}$	-0.33***	0.03	
	r_k	-0.14	0.03	
Linoleic acid (%)	$r_{(M,F,K)}$	0.46***	0.05	-0.40***
	r_k	0.53***	0.06	-0.17
Linolenic acid (%)	$r_{(M,F,K)}$	-0.47***	-0.07	0.33***
	r_k	-0.55***	-0.09	0.12

*significance at p= 0.05, ** significance at p= 0.01, *** significance at p= 0.0001, n.s = not significant. '$r_{(M,F,K)}$' denotes pearson correlation coefficient of pooled mean data of 2006 multi-location field trial; 'r_k' denotes pearson correlation coefficients of mean data of 2005 Kernen Research Farm field trial.

Table A.6. Pearson correlation coefficients of different variables in the 2006 multi-location trial at each location viz., Melfort, Floral and Kernen.

Phenotypic Traits		Fibre concentration (%)	Plant height (cm)	Oil concentration (%)	Protein concentration (%)	Linoleic acid concentration (%)
Plant height (cm)	$r_{(M)}$	-0.10				
	$r_{(F)}$	0.18*				
	$r_{(K)}$	0.09				
Oil concentration (%)	$r_{(M)}$	0.16*	0.01			
	$r_{(F)}$	0.11	0.12			
	$r_{(K)}$	0.21	0.09			
Protein concentration (%)	$r_{(M)}$	-0.08	-0.02	-0.62***		
	$r_{(F)}$	-0.02	-0.02	-0.76***		
	$r_{(K)}$	-0.04	0.01	-0.68***		
Linoleic acid concentration (%)	$r_{(M)}$	-0.19**	-0.28**	0.06	-0.02	
	$r_{(F)}$	-0.20**	-0.31***	-0.06	0.10	
	$r_{(K)}$	-0.20**	-0.26**	-0.03	0.11	
Linolenic acid concentration (%)	$r_{(M)}$	0.18*	0.28**	-0.07	0.03	-0.99***
	$r_{(F)}$	0.20**	0.31***	0.06	-0.10	-0.99***
	$r_{(K)}$	0.18*	0.27**	0.02	-0.10	-0.99***

*significance at p= 0.05, ** significance at p= 0.01, *** significance at p= 0.0001, n.s = not significant.
'$r_{(M)}$', '$r_{(F)}$', '$r_{(K)}$' denotes pearson correlation coefficient of mean data of 2006 multi-location trial in Melfort, Floral and Kernen respectively ; All correlations have been calculated using untransformed data excluding the parents and the checks. All correlations have been calculated using untransformed data excluding the parents and the checks.

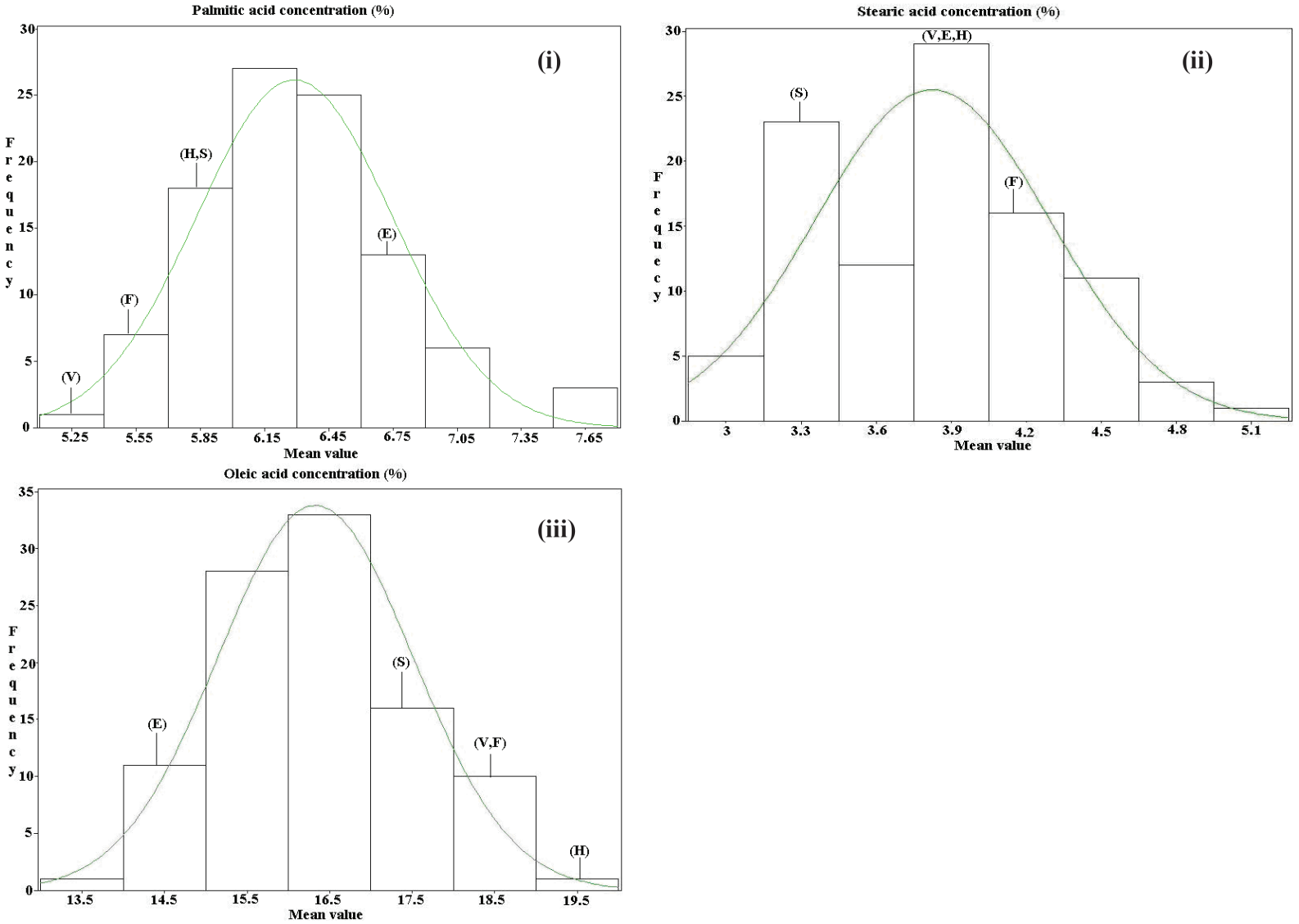

Figure A.1. Histograms and frequency distribution of the RIL population, parents and checks for (i) palmitic acid concentration, (ii) stearic acid concentration and (iii) oleic acid concentration grown in 2006 multi-location field trial. The positions of the parents and the checks on the histograms are represented by their initials viz., Viking (V), E1747 (E), Hermes (H), Flanders (F) and Somme (S).

Table A.7. Mean values of flax fibre and oil related traits of 2005 Kernen Farm trial in Saskatchewan.

SL.No	Acc No.	Oil %	Protein %	Palmitic %	Stearic %	Oleic %	Linoleic %	Linolenic %	Plant Height (cm)	Fibre %
1	**Viking**	**42.33**	**23.10**	**6.23**	**3.00**	**15.86**	**18.00**	**56.91**	**57.33**	**17.78**
2	**E1747**	**41.65**	**23.75**	**6.85**	**3.40**	**12.86**	**74.23**	**2.66**	**45.33**	**15.73**
3	99GH254	39.90	24.40	5.91	3.88	14.43	54.01	21.76	48.33	17.10
4	99GH255	38.40	26.70	5.41	3.95	14.19	20.61	55.85	46.67	18.41
5	99GH256	37.10	27.00	6.75	3.72	15.66	71.31	2.55	41.67	17.41
6	99GH257	37.90	27.30	6.08	2.84	14.36	23.38	53.35	53.67	18.44
7	99GH258	39.50	26.10	5.86	4.18	15.73	35.17	39.05	51.00	19.71
8	99GH259	38.80	26.30	6.04	4.89	13.81	40.39	34.87	40.67	16.31
9	99GH260	38.50	26.60	7.86	3.23	14.03	72.54	2.35	39.33	16.12
10	99GH261	37.40	26.00	6.39	3.06	14.30	74.28	1.98	48.33	17.08
11	99GH263	40.60	23.40	6.05	3.69	15.11	21.46	53.70	57.00	17.68
12	99GH264	39.40	26.10	5.75	2.97	14.42	21.03	55.82	46.67	19.13
13	99GH265	39.60	25.80	6.21	4.08	15.05	21.69	52.97	53.33	19.06
14	99GH266	40.40	25.00	6.22	3.18	14.50	20.16	55.94	54.33	17.16
15	99GH267	37.70	27.10	6.63	4.84	14.85	39.89	33.79	43.33	16.00
16	99GH268	39.90	25.50	5.97	3.89	17.42	21.62	51.10	43.67	19.58
17	99GH269	39.10	25.70	5.96	3.42	13.47	37.83	39.33	51.00	19.40
18	99GH270	41.30	23.20	6.19	3.19	16.35	20.05	54.22	57.67	19.76
19	99GH271	39.90	25.20	7.36	3.06	13.57	73.56	2.45	51.00	15.64
20	99GH272	38.30	26.50	7.02	4.03	14.41	57.09	17.46	54.33	16.98
21	99GH273	39.20	25.50	8.07	3.12	14.81	72.21	1.79	47.00	21.15
22	99GH274	39.20	26.80	6.16	3.24	14.64	22.12	53.84	51.00	21.19
23	99GH275	36.90	27.90	6.52	3.65	14.75	33.49	41.59	47.00	17.75
24	99GH276	40.00	24.70	7.03	3.28	14.20	32.40	43.10	54.67	17.69
25	99GH277	40.20	24.90	6.71	4.85	16.71	33.61	38.12	45.00	18.80
26	99GH278	38.80	26.00	6.12	3.28	15.31	39.56	35.73	55.00	19.63
27	99GH279	38.20	25.90	5.94	3.09	15.60	20.71	54.65	48.67	18.97
28	99GH280	37.70	26.70	6.26	3.87	14.06	40.10	35.71	55.00	19.60
29	99GH281	40.90	25.30	5.96	3.97	14.68	35.01	40.38	58.33	18.94
30	99GH282	39.20	25.20	6.01	4.32	15.74	24.73	49.19	64.33	17.42
31	99GH283	39.40	25.30	6.32	3.87	14.84	19.66	55.31	63.67	17.41

32	99GH284	41.30	24.40	6.59	3.80	14.63	35.12	39.87	70.00	18.31
33	99GH285	39.80	27.10	6.22	3.85	14.03	21.48	54.42	55.67	17.40
34	99GH286	37.50	25.70	6.72	4.19	13.82	40.32	34.96	49.00	17.66
35	99GH287	40.20	25.30	6.04	4.03	14.24	73.49	2.20	63.67	18.80
36	99GH288	40.90	24.70	6.35	3.04	15.43	40.11	35.08	51.67	17.53
37	99GH289	37.80	27.20	6.48	4.03	15.20	33.52	40.77	53.00	18.04
38	99GH290	38.10	25.80	7.12	4.25	14.48	41.69	32.45	52.67	17.20
39	99GH291	40.00	25.90	6.15	3.70	13.70	41.64	34.81	49.00	18.67
40	99GH292	38.70	26.20	6.86	3.34	15.39	72.06	2.34	48.67	17.07
41	99GH293	39.50	25.40	6.47	3.52	16.23	37.96	35.82	57.33	18.02
42	99GH294	39.10	25.50	5.86	2.95	14.57	32.72	43.89	57.67	17.71
43	99GH295	40.80	24.30	6.98	3.24	16.83	59.20	13.74	52.00	16.58
44	99GH296	40.20	24.90	7.12	3.54	13.12	54.38	21.85	59.33	20.29
45	99GH297	40.60	24.60	8.08	4.45	14.06	71.23	2.17	39.00	19.39
46	99GH298	36.20	28.10	6.59	4.03	15.62	71.83	1.93	46.00	19.76
47	99GH299	38.50	26.30	6.30	3.79	14.64	26.44	48.83	53.00	15.92
48	99GH300	40.90	25.10	6.01	3.52	14.01	21.62	54.84	54.67	19.68
49	99GH301	39.70	24.40	6.81	3.74	14.92	34.35	40.18	51.67	16.92
50	99GH302	39.50	25.50	6.42	3.06	14.66	37.23	38.63	57.00	16.76
51	99GH303	38.60	26.60	5.93	3.63	15.06	20.01	55.37	49.00	17.51
52	99GH304	38.90	27.00	7.13	4.79	14.95	41.59	31.55	51.00	18.09
53	99GH305	38.90	26.00	7.11	4.04	12.73	34.21	41.90	54.00	18.40
54	99GH306	39.80	25.60	6.30	2.87	16.62	30.30	43.92	53.00	18.62
55	99GH307	38.70	26.30	6.74	3.52	14.78	72.33	2.64	51.00	17.17
56	99GH308	39.60	26.40	6.95	4.05	14.66	72.27	2.07	56.67	17.82
57	99GH309	38.70	26.60	6.60	5.17	14.32	71.81	2.11	55.00	19.13
58	99GH310	37.50	27.50	6.25	4.09	13.65	41.09	34.92	54.33	18.68
59	99GH311	39.90	25.80	6.44	3.93	15.39	71.99	2.25	50.00	20.56
60	99GH312	37.80	26.70	5.73	4.07	15.55	23.06	51.58	57.67	18.79
61	99GH313	40.20	24.40	6.45	3.91	15.38	37.70	36.57	48.00	17.35
62	99GH314	38.50	26.40	5.71	4.59	14.79	33.43	41.49	49.00	16.62
63	99GH315	39.90	25.40	6.85	4.70	14.75	39.85	33.84	44.33	18.39
64	99GH316	37.00	27.00	7.15	3.24	13.86	35.44	40.31	51.33	19.80
65	99GH317	38.90	25.90	7.28	3.30	14.79	38.15	36.47	52.00	17.37
66	99GH318	39.20	25.00	6.45	3.22	17.06	39.10	34.17	60.00	19.81

67	99GH319	40.30	25.50	6.25	4.35	15.37	71.64	2.40	52.67	19.97
68	99GH320	40.90	25.20	6.65	4.17	14.68	33.68	40.82	63.00	18.94
69	99GH321	38.70	27.20	6.06	3.42	13.61	34.00	42.91	52.00	17.09
70	99GH322	39.70	25.60	7.37	4.23	15.00	69.71	3.69	63.00	18.11
71	99GH323	39.20	25.80	6.57	3.31	13.52	55.74	20.87	52.00	19.10
72	99GH324	40.60	23.40	5.78	4.36	14.12	24.16	51.59	62.33	17.44
73	99GH325	39.50	26.00	6.68	3.73	15.20	33.65	40.73	50.67	17.71
74	99GH326	38.40	26.70	5.74	4.22	15.09	53.92	21.03	50.00	16.30
75	99GH327	39.20	26.10	6.27	3.67	11.22	76.12	2.72	54.00	17.87
76	99GH328	39.30	26.00	5.85	3.55	13.81	24.51	52.28	58.00	19.37
77	99GH329	38.60	26.20	6.62	3.37	14.93	23.01	52.08	56.33	19.76
78	99GH330	40.50	25.20	6.98	3.53	13.15	32.85	43.48	56.33	19.87
79	99GH332	40.30	23.90	6.47	3.37	14.75	41.43	33.99	60.67	17.20
80	99GH333	38.60	26.70	7.56	4.06	12.98	72.90	2.50	46.33	15.97
81	99GH334	37.90	26.20	6.51	3.44	13.59	22.83	53.64	49.67	19.05
82	99GH335	38.80	26.70	7.42	3.00	13.42	73.46	2.71	56.67	17.52
83	99GH336	39.10	26.50	5.89	4.04	15.06	53.95	21.05	63.00	20.59
84	99GH337	38.60	26.00	6.98	3.30	14.39	40.37	34.97	41.67	20.43
85	99GH338	36.50	27.40	6.79	4.79	15.03	70.73	2.65	45.67	16.97
86	99GH339	38.60	26.40	6.62	4.16	16.15	51.33	21.75	43.67	19.92
87	99GH340	40.10	24.80	6.06	4.33	14.91	24.06	50.64	57.00	18.42
88	99GH341	39.10	25.20	6.76	3.71	15.68	24.00	49.85	50.67	23.46
89	99GH342	37.20	27.80	6.65	3.42	14.51	72.44	2.99	51.67	18.22
90	99GH343	37.80	26.20	6.18	4.45	13.41	21.37	54.58	53.33	17.82
91	99GH344	39.10	25.60	5.47	4.32	13.58	33.62	43.01	55.67	18.91
92	99GH345	39.70	24.00	6.84	3.42	14.52	72.29	2.93	54.67	17.82
93	99GH346	38.60	24.90	6.55	4.37	14.15	37.38	37.56	49.33	19.72
94	99GH347	38.30	25.00	6.42	4.58	16.28	34.21	38.52	43.00	16.78
95	99GH348	38.50	26.10	6.76	3.12	14.74	37.69	37.70	50.00	18.94
96	99GH349	40.70	24.90	6.65	3.30	14.81	33.96	41.28	47.67	18.87
97	99GH350	36.10	27.40	5.75	3.71	15.86	19.93	54.76	63.33	21.02

Table A.8. Pooled mean values of flax fibre and oil related traits in 2006 multi-location field trial at Kernen, Melfort and Floral locations in Saskatchewan.

Sl.No	Accession	Fibre %	Plant height (cm)	Oil %	Protein %	Palmitic acid %	Stearic acid %	Oleic acid %	Linoleic acid %	Linolenic acid %
1	**Viking**	19.64	72.00	38.00	26.20	5.24	3.91	18.74	16.78	55.04
2	**E1747**	15.64	55.33	41.15	24.85	6.84	3.85	14.59	69.31	4.38
3	**Hermes**	19.87	86.08	38.00	27.28	5.60	3.84	19.57	19.61	52.13
4	**Flanders**	17.36	57.58	42.60	23.48	5.52	4.15	18.06	15.94	56.25
5	**CDC Somme**	15.30	60.08	41.83	24.50	5.86	3.22	17.83	15.11	57.77
6	99GH254	17.62	60.00	39.90	25.13	5.88	3.95	16.41	50.37	23.40
7	99GH255	17.01	57.17	37.90	27.22	5.56	4.13	16.44	18.87	54.94
8	99GH256	17.38	49.67	38.20	26.60	6.33	3.67	15.84	70.66	3.00
9	99GH257	18.34	61.67	38.18	27.25	6.19	3.28	16.96	22.12	52.12
10	99GH258	19.13	57.50	38.93	27.13	5.80	4.07	16.34	33.98	39.70
11	99GH259	17.95	51.25	38.30	26.78	6.08	4.82	17.01	38.31	33.78
12	99GH260	16.31	47.17	39.57	25.75	7.55	3.22	15.43	69.63	4.18
13	99GH261	17.30	54.50	38.13	25.43	6.34	3.21	16.14	71.11	3.37
14	99GH263	19.12	62.00	40.13	24.45	6.12	3.82	17.07	20.48	52.50
15	99GH264	20.78	55.08	39.78	26.03	5.70	2.86	18.36	20.64	52.45
16	99GH265	20.06	67.00	39.93	25.55	6.03	3.94	16.65	19.90	53.48
17	99GH266	17.73	64.33	39.55	25.50	6.27	3.32	17.05	18.71	54.65
18	99GH267	16.22	53.92	38.25	26.85	6.14	4.58	15.75	36.44	37.09
19	99GH268	19.92	51.92	40.10	25.93	5.68	3.68	18.22	20.11	52.31
20	99GH269	17.86	58.17	39.18	26.55	5.85	3.29	14.53	35.72	40.62

Sl.No	Accession	Fibre %	Plant height (cm)	Oil %	Protein %	Palmitic acid %	Stearic acid %	Oleic acid %	Linoleic acid %	Linolenic acid %
21	99GH270	19.14	64.67	40.42	24.02	5.97	3.29	18.91	18.41	53.42
22	99GH271	15.56	57.50	39.80	25.40	7.15	3.11	14.11	72.75	3.05
23	99GH272	16.17	60.00	38.53	26.68	6.79	3.78	15.34	47.24	25.19
24	99GH273	18.96	55.75	39.03	26.28	7.50	3.12	14.93	71.74	2.71
25	99GH274	19.11	59.17	38.90	27.30	6.44	3.36	17.56	20.23	52.40
26	99GH275	17.06	58.00	37.80	27.33	6.44	3.69	15.39	29.72	44.76
27	99GH276	16.80	61.83	39.33	25.53	6.95	3.29	15.72	29.85	44.19
28	99GH277	18.37	52.42	40.67	25.03	6.75	4.39	18.23	30.66	39.98
29	99GH278	18.44	60.08	39.65	26.03	5.88	3.23	16.88	35.91	38.11
30	99GH279	18.03	52.67	38.33	26.58	5.81	3.18	18.31	20.15	52.55
31	99GH280	18.44	58.42	37.03	27.60	5.89	3.84	16.49	38.13	35.65
32	99GH281	16.97	58.00	40.62	25.90	6.21	4.21	16.14	32.46	40.99
33	99GH282	16.98	63.83	38.72	26.17	6.29	4.03	18.10	24.81	46.77
34	99GH283	17.65	68.75	39.20	26.13	6.29	3.83	16.36	17.91	55.61
35	99GH284	18.38	72.25	40.80	25.18	6.43	3.84	15.67	34.06	40.01
36	99GH285	18.17	59.33	39.95	27.73	6.15	3.99	15.57	19.79	54.50
37	99GH286	17.22	55.75	38.05	25.68	6.48	4.13	14.39	38.66	36.35
38	99GH287	18.28	62.42	40.43	25.90	6.01	4.11	15.33	69.65	4.91
39	99GH288	17.83	58.08	40.93	25.52	6.25	3.19	16.56	38.51	35.50
40	99GH289	18.57	58.75	38.70	27.37	6.31	3.98	16.56	32.20	40.95
41	99GH290	16.79	54.58	38.12	26.25	6.67	4.28	15.75	40.45	32.87
42	99GH291	17.68	51.58	40.68	25.85	6.00	3.84	15.31	41.58	33.27
43	99GH292	16.57	57.17	39.47	25.85	6.46	3.55	16.56	69.23	4.20

Sl.No	Accession	Fibre %	Plant height (cm)	Oil %	Protein %	Palmitic acid %	Stearic acid %	Oleic acid %	Linoleic acid %	Linolenic acid %
44	99GH293	16.86	67.83	39.00	25.92	6.09	3.80	18.15	36.49	35.47
45	99GH294	17.76	63.08	39.12	26.45	5.79	3.16	15.50	30.73	44.82
46	99GH295	16.56	57.00	40.80	25.13	6.99	3.56	18.16	51.81	19.49
47	99GH296	19.22	65.33	40.28	25.50	7.00	3.86	15.11	53.55	20.49
48	99GH297	17.89	43.92	40.48	25.08	7.59	4.49	14.75	69.22	3.96
49	99GH298	17.69	49.50	37.37	27.93	6.20	3.92	15.63	69.69	3.56
50	99GH299	16.28	64.00	38.85	26.43	6.06	4.11	16.29	22.46	51.09
51	99GH300	18.60	63.25	40.40	26.23	5.82	3.67	15.14	19.91	55.47
52	99GH301	16.09	61.50	39.53	25.63	6.55	3.81	15.95	32.10	41.58
53	99GH302	16.34	62.25	38.78	26.72	6.20	3.24	17.07	34.03	39.47
54	99GH303	17.35	61.00	39.43	26.83	5.75	3.71	17.71	17.55	55.28
55	99GH304	16.88	59.58	40.08	26.83	6.79	4.53	16.01	40.75	31.92
56	99GH305	19.35	61.83	38.92	26.13	6.84	4.01	14.14	32.16	42.85
57	99GH306	20.31	58.58	40.07	25.53	6.13	3.15	17.96	26.09	46.68
58	99GH307	16.14	59.42	38.40	27.30	6.14	3.67	16.33	69.93	3.95
59	99GH308	17.31	60.67	40.42	26.10	6.59	3.97	16.89	70.55	2.00
60	99GH309	18.40	61.75	39.07	27.27	6.13	5.11	15.91	70.66	2.19
61	99GH310	18.31	63.00	37.75	27.82	6.54	4.13	15.76	40.67	32.90
62	99GH311	19.34	59.92	40.48	25.92	6.32	4.03	16.22	68.84	4.58
63	99GH312	17.91	63.08	39.02	26.88	5.67	3.94	17.27	21.74	50.87
64	99GH313	18.47	54.58	40.65	24.72	6.55	3.93	16.65	38.57	34.30
65	99GH314	17.33	59.50	38.67	26.78	5.85	4.34	15.99	30.97	42.68
66	99GH315	18.77	57.25	39.93	25.83	6.61	4.40	15.75	47.10	24.48

Sl.No	Accession	Fibre %	Plant height (cm)	Oil %	Protein %	Palmitic acid %	Stearic acid %	Oleic acid %	Linoleic acid %	Linolenic acid %
67	99GH316	18.92	60.33	37.47	27.03	6.84	3.32	15.83	33.72	40.30
68	99GH317	16.93	58.75	39.88	25.53	6.61	3.35	15.05	35.60	39.39
69	99GH318	18.20	65.67	39.35	25.38	6.36	3.25	17.85	38.29	34.25
70	99GH319	19.64	55.58	40.00	26.75	6.11	4.29	16.07	69.42	3.78
71	99GH320	17.67	67.67	40.72	26.23	6.38	4.17	16.12	31.26	42.06
72	99GH321	17.68	59.58	38.93	27.63	6.19	3.79	14.77	32.31	42.94
73	99GH322	16.26	65.25	39.38	26.42	7.07	4.38	15.96	68.48	4.11
74	99GH323	18.84	55.25	39.90	26.20	6.41	3.28	16.27	53.75	21.97
75	99GH324	15.90	66.58	40.42	24.70	5.55	4.65	17.21	24.14	48.45
76	99GH325	17.58	56.58	39.17	26.40	6.73	4.11	16.56	32.35	40.25
77	99GH326	16.71	55.08	38.77	27.45	5.92	4.70	16.88	53.54	18.97
78	99GH327	17.41	54.00	39.45	26.10	6.30	4.20	13.42	73.38	2.70
79	99GH328	18.60	65.67	38.97	26.80	5.78	3.83	16.38	22.46	51.56
80	99GH329	17.92	58.92	38.48	26.45	6.20	3.31	15.23	24.87	50.39
81	99GH330	18.85	59.67	40.20	25.93	6.70	3.70	16.47	33.69	39.44
82	99GH332	17.26	61.50	39.58	25.52	6.10	3.76	16.13	36.49	37.53
83	99GH333	17.40	55.75	39.12	26.55	7.07	3.86	14.58	71.36	3.14
84	99GH334	17.40	52.33	38.05	26.98	6.31	3.62	16.03	21.10	52.94
85	99GH335	17.08	60.83	37.90	28.23	6.70	3.03	15.13	69.18	4.64
86	99GH336	20.16	65.67	38.38	27.98	5.98	4.26	18.00	55.69	16.06
87	99GH337	20.31	50.83	39.75	25.32	6.80	3.43	15.74	39.34	34.69
88	99GH338	17.28	57.08	37.30	27.23	6.46	4.51	14.37	69.15	5.53
89	99GH339	19.05	51.08	39.22	26.67	6.48	3.94	15.73	55.41	18.27

Sl.No	Accession	Fibre %	Plant height (cm)	Oil %	Protein %	Palmitic acid %	Stearic acid %	Oleic acid %	Linoleic acid %	Linolenic acid %
90	99GH340	17.74	67.08	39.85	25.78	5.94	4.15	17.23	22.11	50.58
91	99GH341	21.13	59.83	39.87	25.30	6.13	3.63	17.58	21.40	51.26
92	99GH342	16.69	61.50	37.13	28.10	6.54	3.44	16.31	70.67	3.04
93	99GH343	17.12	61.58	37.85	26.62	6.00	4.47	14.48	18.39	56.66
94	99GH344	18.78	71.92	39.22	26.57	5.47	4.61	16.03	31.27	42.58
95	99GH345	17.46	59.42	40.02	24.83	6.39	3.39	16.07	70.74	3.00
96	99GH346	18.15	52.00	38.15	26.43	6.26	4.56	15.95	35.19	38.05
97	99GH347	17.61	51.00	38.92	25.13	6.49	4.47	16.91	32.09	40.05
98	99GH348	19.03	53.25	37.83	27.13	6.54	3.19	17.03	37.49	35.74
99	99GH349	18.21	58.17	39.80	26.27	6.54	3.54	16.70	31.81	41.25
100	99GH350	20.52	68.42	36.52	27.98	5.72	3.76	17.35	18.42	54.61

Appendix B

Table B.1. Pearson correlation coefficients between anatomical and multi-location field traits (plant height and NIR fibre content) at the seedling stage.

Anatomical traits		Fibre: Phloem	Fibre: Xylem	Xylem: Stem	Pith: Stem	Plant ht	NIR Fibre
Epiw	f	-0.23	-0.01	-0.01	0.02	-0.17	-0.36
	p	0.68	-0.51	0.33*	0.48**	0.08	-0.16
Cortw	f	0.17	0.27	-0.09	-0.33	0.13	0.43*
	p	0.16	-0.01	0.18	0.04	0.11	0.26
Camw	f	-0.11	-0.09	0.11	0.16	-0.26	-0.16
	p	0.08	-0.19	0.13	0.48**	0.07	-0.13
Avgfarea	f	0.23	0.54**	-0.19	0.06	0.30	0.45**
	p	0.19	-0.06	-0.14	0.07	0.23	0.51**
No. fibre	f	0.09	0.30	0.08	-0.18	0.01	0.408*
	p	0.25	0.69***	0.02	-0.20	-0.19	0.26
Stem area	f	0.17	0.34	-0.08	-0.38*	0.05	0.31
	p	-0.56**	-0.10	-0.53	-0.08	0.05	0.32
Tot.fibre area	f	0.41*	0.68***	-0.02	-0.28	0.09	0.48**
	p	0.27	0.82***	-0.01	-0.04	-0.06	0.48**
Pith area	f	0.02	0.20	0.00	0.61**	-0.03	0.05
	p	-0.03	-0.35*	0.24	0.75***	-0.18	-0.10
Xylem area	f	0.22	0.02	0.45**	-0.29	0.02	0.22
	p	0.18	-0.44	0.60**	0.68***	-0.06	-0.07
Phloem area	f	-0.39*	0.36*	-0.19	-0.29	0.06	-0.01
	p	-0.71***	0.19	-0.61**	-0.27	0.11	0.37*
Fibre: Stem	f	0.57**	0.82***	0.05	-0.04	0.09	0.52**
	p	0.52**	0.80***	0.25	-0.02	-0.05	0.27
Fibre: Phloem	f		0.41*	0.12	-0.11	0.07	0.64***
	p		0.16	0.65***	0.40*	-0.18	-0.09
Fibre: Xylem	f			-0.52**	-0.04	0.10	0.51**
	p			-0.30	-0.38	-0.01	0.48**
Xylem: Stem	f				0.09	-0.04	-0.17
	p				0.72	-0.11	-0.34
Pith: Stem	f					-0.05	-0.22
	p					-0.22	-0.38

p= growth chamber, f= field *significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$.

The anatomical regions are denoted by: Epidermis width (Epiw), cortex width (Cortw), cambial zone width (Camw), average area of single fibre cell (Avg.fc area), number of fibre cells (No. fibre), total fibre area in the stem (Tot.fibre area), fibre to stem area ratio (Fibre : Stem), fibre to phloem area ratio (Fibre: Phloem), fibre to xylem area ratio (Fibre : Xylem), xylem to stem area ratio (Xylem : Stem) and pith to stem area ratio (Pith: Stem).

Table B.2. Pearson correlation coefficients between anatomical and multi-location field traits (plant height and NIR fibre content) at the green capsule stage.

Anatomical traits		Fibre: Phloem	Fibre: Xylem	Xylem: Stem	Pith: Stem	Plant ht	NIR Fibre
Epiw	**f**	0.19	0.30	0.24	-0.27	0.28	0.40*
	p	-0.08	0.05	0.11	-0.08	0.29	0.05
Cortw	**f**	-0.14	-0.16	-0.42*	0.03	-0.26	-0.21
	p	-0.05	-0.18	0.14	0.01	-0.07	0.13
Camw	**f**	0.16	0.27	0.35	-0.43*	0.07	0.10
	p	0.20	0.21	0.11	0.08	0.39	0.32
Avgfarea	**f**	0.23	0.52*	0.30	-0.23	0.12	0.63***
	p	0.19	0.74***	-0.42*	0.08	0.11	0.61***
No. fibre	**f**	0.51**	0.43*	0.25	-0.06	0.08	0.37*
	p	0.02	0.58**	-0.38*	0.38*	-0.07	0.36*
Stem area	**f**	0.34	0.32	0.48**	-0.32	0.31	0.46**
	p	0.18	0.463**	-0.35*	0.02	0.29	0.40
Tot.fibre area	**f**	0.56**	0.79***	0.28	-0.12	0.04	0.55**
	p	0.24	0.89***	-0.16	0.19	0.12	0.47**
Pith area	**f**	0.69***	0.40*	0.42*	0.28	0.19	0.53**
	p	0.33	0.46**	-0.24	0.71***	0.02	0.40*
Xylem area	**f**	0.38*	0.25	0.63***	-0.31	0.37*	0.46**
	p	0.34	0.01	0.45**	0.04	0.41*	-0.01
Phloem area	**f**	0.07	0.54**	0.14	-0.47**	0.16	0.51**
	p	-0.28	0.69***	-0.24	0.06	0.16	0.41*
Fibre: Stem	**f**	0.63***	0.98***	0.08	0.10	-0.15	0.46**
	p	0.19	0.85***	-0.03	0.22	-0.02	0.38*
Fibre: Phloem	**f**		0.56**	0.37*	0.54**	-0.08	0.31
	p		0.10	0.22	0.25	0.18	0.11
Fibre: Xylem	**f**			-0.11	0.14	-0.23	0.40*
	p			-0.54**	0.21	-0.09	0.59
Xylem: Stem	**f**				-0.17	0.39*	0.29
	p				-0.09	0.16	-0.50*
Pith: Stem	**f**					-0.23	0.08
	p					-0.24	0.20

p= growth chamber, f= field *significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$.

The anatomical regions are denoted by: Epidermis width (Epiw), cortex width (Cortw), cambial zone width (Camw), average area of single fibre cell (Avg.fc area), number of fibre cells (No. fibre), total fibre area in the stem (Tot.fibre area), fibre to stem area ratio (Fibre : Stem), fibre to phloem area ratio (Fibre: Phloem), fibre to xylem area ratio (Fibre : Xylem), xylem to stem area ratio (Xylem : Stem) and pith to stem area ratio (Pith: Stem).

Table B.3. Pearson correlation coefficients between anatomical and multi-location field traits (plant height and NIR fibre content) at the physiological maturity stage.

Anatomical traits		Fibre: Stem	Fibre: Phloem	Fibre: Xylem	Xylem: Stem	Pith: Stem	Plant ht	NIR Fibre
Epiw	**f**	-0.03	0.09	-0.06	0.02	-0.15	-0.10	-0.17
	p	0.10	-0.17	0.11	-0.03	-0.42*	0.00	0.19
Cortw	**f**	0.30	0.23	0.39*	-0.43*	0.10	-0.33	-0.12
	p	-0.10	0.14	-0.05	-0.20	0.19	-0.13	0.09
Camw	**f**	0.07	0.31	-0.02	0.18	-0.23	0.22	0.17
	p	0.24	0.20	0.22	0.04	-0.51**	0.35*	0.54**
Avgfarea	**f**	0.40*	0.36*	0.22	0.44**	-0.27	0.28	0.55**
	p	0.72***	0.36*	0.69***	-0.25	-0.35*	0.38*	0.50**
No. fibre	**f**	0.77***	0.65***	0.61***	0.06	-0.06	0.07	0.41*
	p	0.74***	0.56**	0.73	-0.39*	-0.23	-0.08	0.55**
Stem area	**f**	0.28	0.42*	-0.01	0.69***	-0.35*	0.45**	0.27
	p	0.27	0.05	0.24	0.15	-0.27	0.55**	0.15
Tot.fibre area	**f**	0.77***	0.70***	0.53**	0.31	-0.18	0.14	0.45**
	p	0.92***	0.53**	0.89***	-0.36	-0.29	0.16	0.51**
Pith area	**f**	0.34	0.56**	0.20	0.27	0.30	0.34	0.20
	p	0.10	-0.05	0.13	-0.04	0.37	0.17	-0.24
Xylem area	**f**	0.13	0.34	-0.20	0.84***	-0.46**	0.54**	0.26
	p	-0.01	0.00	-0.10	0.54**	-0.34	0.69***	0.04
Phloem area	**f**	0.48**	0.14	0.30	0.30	-0.39*	0.04	0.24
	p	0.66***	0.05	0.70***	-0.41*	-0.28	0.25	0.36*
Fibre: Stem	**f**		0.69***	0.92***	-0.19	0.04	-0.18	0.54**
	p		0.70***	0.96***	-0.46**	-0.26	-0.06	0.59**
Fibre: Phloem	**f**			0.51**	0.18	0.17	0.22	0.49**
	p			0.58**	0.01	-0.19	-0.05	0.46**
Fibre: Xylem	**f**				-0.542**	0.26	-0.32	0.42*
	p				-0.62**	-0.14	-0.18	0.53**
Xylem: Stem	**f**					-0.57**	0.56**	0.16
	p					-0.33	0.50	-0.15
Pith: Stem	**f**						-0.05	-0.05
	p						-0.55**	-0.68***

p= growth chamber, f= field *significance at $p < 0.05$, **significance at $p < 0.01$, *** significance at $p < 0.0001$. The anatomical regions are denoted by: Epidermis width (Epiw), cortex width (Cortw), cambial zone width (Camw), average area of single fibre cell (Avg.fc area), number of fibre cells (No. fibre), total fibre area in the stem (Tot.fibre area), fibre to stem area ratio (Fibre : Stem), fibre to phloem area ratio (Fibre: Phloem), fibre to xylem area ratio (Fibre : Xylem), xylem to stem area ratio (Xylem : Stem) and pith to stem area ratio (Pith: Stem).

Table B.4.1. Mean of anatomical traits for selected 14 genotypes at the seedling stage. (*Mean ± Standard Error)

Seedling Field	Epiw (µm)	Cortw (µm)	Camw (µm)	Avg. fc area (μm^2)	No. fibre
Viking	18.8 ±0.2	54.4 ±2.6	93.6 ±4.2	856.8 ±44.2	292.0 ±36.7
Hermes	18.4 ±1.1	51.4 ±2.0	85.8 ±9.7	1012.8 ±145.3	193.0 ±26.1
E1747	15.2 ±0.5	42.2 ±4.4	81.6 ±2.5	646.6 ±94.2	150.0 ±16.7
H337	13.0 ±0.5	53.5 ±1.2	81.7 ±6.1	535.7 ±61.1	334.0 ±60.5
H341	13.8 ±0.7	49.3 ±4.0	55.0 ±1.9	533.2 ±48.8	236.0 ±11.9
H350	14.2 ±1.6	47.9 ±1.7	70.7 ±7.9	682.6 ±34.8	305.0 ±7.8
L260	15.3 ±0.6	40.0 ±1.2	70.3 ±5.1	411.1 ±28.6	214.0 ±29.0
L267	15.5 ±0.2	44.3 ±3.2	68.9 ±8.8	494.7 ±93.3	235.0 ±28.3
L271	15.3 ±1.1	42.9 ±1.6	70.3 ±3.8	479.0 ±46.8	191.0 ±7.0
D273	15.2 ±0.5	51.2 ±5.6	64.1 ±9.7	577.8 ±78.8	272.0 ±36.2
D311	15.7 ±0.5	54.3 ±2.9	68.8 ±3.4	683.3 ±85.1	300.0 ±50.5
D319	13.9 ±0.8	46.7 ±6.5	53.3 ±4.4	619.4 ±53.9	364.0 ±45.2
S268	15.0 ±0.5	44.5 ±0.2	60.7 ±5.0	851.3 ±59.6	204.0 ±33.5
T284	13.7 ±0.7	45.9 ±4.8	54.5 ±6.3	677.3 ±72.4	212.0 ±27.4

Seedling Growth chamber	Epiw (µm)	Cortw (µm)	Camw (µm)	Avg. fc area (μm^2)	No. fibre
Viking	15.9 ±0.6	40.6 ±2.5	77.1 ±4.2	666.6 ±77.9	129.0 ±20.9
Hermes	18.7 ±1.7	49.6 ±1.8	80.1 ±14.1	830.6 ±162.5	151.0 ±13.5
E1747	18.9 ±3.2	37.0 ±4.9	66.6 ±4.1	371.1 ±37.5	154.0 ±29.0
H337	16.4 ±2.0	50.7 ±2.1	70.3 ±1.4	694.1 ±53.9	236.0 ±28.8
H341	15.1 ±1.4	50.9 ±7.1	51.5 ±2.5	558.3 ±143.8	146.0 ±19.6
H350	15.4 ±2.3	45.7 ±6.0	71.7 ±5.4	682.2 ±14.5	151.0 ±7.6
L260	14.6 ±1.6	42.3 ±2.0	59.8 ±0.4	399.7 ±3.8	140.0 ±12.3
L267	19.1 ±1.7	45.1 ±3.2	75.0 ±5.4	407.4 ±16.8	123.0 ±11.0
L271	17.2 ±0.3	42.1 ±4.0	62.4 ±1.4	408.0 ±72.2	155.0 ±3.5
D273	15.6 ±1.9	41.9 ±2.6	56.8 ±4.3	496.4 ±58.0	190.0 ±25.6
D311	13.5 ±0.6	48.2 ±8.0	54.8 ±3.1	480.5 ±38.7	306.0 ±28.8
D319	16.3 ±0.8	49.2 ±7.0	58.2 ±4.1	447.7 ±19.9	196.0 ±15.8
S268	19.1 ±2.5	40.7 ±4.1	71.0 ±2.7	914.4 ±81.2	127.0 ±21.5
T284	19.3 ±0.6	46.7 ±2.0	68.5 ±4.7	727.8 ±86.9	87.0 ±22.6

Table B.4.2. Mean of anatomical traits (Part A) for selected 14 genotypes at the green capsule stage. (*Mean ± Standard Error)

GC Field	Epiw (µm)	Cortw (µm)	Camw (µm)	Avg. fc area (μm^2)	No. Fibre
Viking	14.0 ±0.7	33.3 ±1.1	49.5 ±5.0	1143.4 ±109.2	700.0 ±127.8
Hermes	17.6 ±1.3	35.9 ±6.7	45.6 ±8.7	1178.5 ±92.0	803.0 ±95.5
E1747	18.9 ±2.7	36.4 ±2.6	54.9 ±3.2	605.4 ±122.2	648.0 ±18.5
H337	15.5 ±0.7	30.7 ±2.3	43.2 ±7.3	906.5 ±96.3	501.0 ±79.4
H341	17.8 ±0.9	26.4 ±3.7	51.3 ±7.0	1089.9 ±174.8	648.0 ±186.0
H350	16.2 ±0.2	23.8 ±1.8	42.0 ±3.9	886.3 ±38.8	481.0 ±41.6
L260	13.0 ±1.8	33.8 ±4.9	44.8 ±8.2	618.0 ±46.6	410.0 ±47.0
L267	14.3 ±1.3	37.0 ±3.4	35.3 ±3.0	577.4 ±38.3	300.0 ±12.6
L271	15.3 ±0.8	38.5 ±8.6	49.2 ±7.6	546.3 ±65.9	503.0 ±57.0
D273	14.1 ±1.2	47.5 ±4.6	29.4 ±2.8	540.3 ±30.6	538.0 ±71.0
D311	13.5 ±1.0	49.8 ±9.0	33.0 ±3.7	604.2 ±82.2	477.0 ±120.8
D319	17.6 ±1.9	39.9 ±3.4	54.3 ±5.5	973.3 ±149.2	653.0 ±200.8
S268	18.6 ±0.7	30.6 ±2.3	51.7 ±2.3	1166.9 ±99.0	625.0 ±97.1
T284	17.7 ±1.3	25.8±3.3	51.2 ±4.2	876.9 ±47.2	531.0 ±36.3

GC Growth chamber	Epiw (µm)	Cortw (µm)	Camw (µm)	Avg. fc area (μm^2)	No. Fibre
Viking	13.8 ±0.7	40.6 ±3.8	57.4 ±7.4	979.9 ±104.4	772.0 ±111.3
Hermes	17.0 ±1.0	43.2 ±2.1	58.9 ±8.1	1306.6 ±219.6	773.0 ±54.5
E1747	15.5 ±1.5	35.7 ±2.7	57.2 ±3.0	486.2 ±8.7	548.0 ±39.4
H337	14.7 ±0.8	41.5 ±3.4	59.3 ±3.9	625.9 ±27.6	650.0 ±63.0
H341	13.6 ±0.6	31.4 ±1.7	47.6 ±3.4	704.5 ±31.4	569.0 ±32.4
H350	13.8 ±1.3	32.6 ±2.3	57.4 ±3.4	784.2 ±141.6	628.0 ±5.6
L260	12.7 ±0.2	32.4 ±5.9	43.0 ±1.6	535.8 ±35.7	589.0 ±52.2
L267	14.5 ±0.6	38.5 ±4.6	50.7 ±3.8	457.5 ±9.4	414.0 ±19.4
L271	12.9 ±1.5	33.9 ±4.8	47.8 ±6.3	367.9 ±64.7	503.0 ±70.4
D273	12.6 ±1.4	54.5 ±3.6	52.2 ±3.8	419.1 ±13.9	395.0 ±36.5
D311	12.4 ±1.2	62.0 ±5.0	59.7 ±5.8	459.4 ±40.4	556.0 ±67.4
D319	13.4 ±0.8	44.4 ±2.3	53.5 ±5.3	589.9 ±25.7	635.0 ±27.7
S268	13.4 ±0.7	36.9 ±7.6	67.9 ±5.7	846.9 ±81.8	541.0 ±60.2
T284	16.2 ±0.7	34.6 ±3.3	71.9 ±0.9	559.9 ±27.1	477.0 ±56.7

Table B.4.3. Mean of anatomical traits (Part A) for selected 14 genotypes at physiological maturity stage. (*Mean ± Standard Error)

Maturity Field	Epiw (µm)	Cortw (µm)	Camw (µm)	Avg. fc area (μm^2)	No. Fibre
Viking	13.5 ±2.2	38.8 ±5.4	52.7 ±5.2	1245.6 ±32.4	765.0 ±56.2
Hermes	15.0 ±0.9	36.7 ±4.1	56.8 ±6.0	1191.7 ±111.2	911.0 ±184.5
E1747	18.0 ±1.1	39.4 ±7.0	62.3 ±1.9	742.3 ±115.3	689.0 ±53.7
H337	14.8 ±1.8	42.4 ±2.6	48.1 ±4.4	966.5 ±191.5	432.0 ±33.6
H341	14.0 ±1.2	34.0 ±6.7	42.1 ±6.9	719.7 ±63.0	538.0 ±66.2
H350	14.5 ±1.7	32.2 ±6.3	47.9 ±1.8	960.1 ±158.2	572.0 ±111.2
L260	16.7 ±1.6	50.5 ±9.2	41.8 ±3.4	509.4 ±56.6	453.0 ±71.0
L267	16.8 ±0.9	44.7 ±0.3	46.7 ±2.9	557.6 ±63.9	340.0 ±59.9
L271	14.2 ±1.2	44.0 ±3.2	41.5 ±5.0	447.8 ±76.0	343.0 ±44.0
D273	12.2 ±0.3	62.8 ±3.3	32.8 ±2.3	675.1 ±49.3	449.0 ±72.6
D311	14.9 ±1.0	74.2 ±2.4	45.2 ±4.0	807.0 ±73.9	847.0 ±67.1
D319	16.5 ±0.9	39.6 ±3.9	58.8 ±4.4	923.0 ±110.0	628.0 ±111.4
S268	16.8 ±1.3	51.7 ±7.1	61.6 ±5.2	1269.9 ±72.9	483.0 ±39.3
T284	16.5 ±1.6	32.9 ±3.9	63.4 ±13.9	1114.9 ±247.1	344.0 ±78.6

Maturity Growth chamber	Epiw (µm)	Cortw (µm)	Camw (µm)	Avg. fc area (μm^2)	No. fibre
Viking	14.8 ±1.7	24.9 ±3.2	56.9 ±1.4	1522.7 ±193.5	702.0 ±65.3
Hermes	19.3 ±0.8	29.8 ±5.5	52.3 ±2.5	2198.5 ±565.4	737.0 ±32.0
E1747	16.8 ±0.4	25.3 ±0.9	61.8 ±2.3	601.2 ±48.2	514.0 ±41.8
H337	15.3 ±0.3	30.6 ±2.8	51.3 ±1.4	868.5 ±172.8	673.0 ±83.8
H341	14.1 ±1.3	23.9 ±3.0	49.2 ±0.9	700.4 ±35.3	604.0 ±6.2
H350	14.7 ±1.0	25.3 ±2.0	53.8 ±0.8	1197.2 ±192.2	671.0 ±24.3
L260	13.9 ±1.2	31.5 ±3.2	44.9 ±2.7	518.4 ±35.9	568.0 ±61.8
L267	13.1 ±0.7	30.5 ±1.5	41.7 ±2.5	484.4 ±75.7	521.0 ±19.3
L271	14.0 ±0.8	21.2 ±0.5	46.8 ±1.7	391.6 ±16.7	302.0 ±43.1
D273	15.5 ±1.3	33.2 ±1.7	50.9 ±1.7	483.3 ±98.9	459.0 ±105.1
D311	13.0 ±0.8	36.2 ±5.7	55.6 ±2.4	563.4 ±6.9	560.0 ±95.2
D319	13.7 ±1.4	42.4 ±4.6	54.2 ±7.0	619.4 ±115.2	598.0 ±24.0
S268	14.8 ±0.6	26.7 ±1.9	53.9 ±0.3	1176.6 ±94.0	656.0 ±99.2
T284	14.4 ±1.1	30.6 ±1.5	53.8 ±1.3	1060.9 ±281.1	481.0 ±3.9

Table B.4.4. Mean of anatomical traits (Part B) for selected 14 genotypes at seedling stage. (*Mean ± Standard Error)

Seedling Field	Stem area (mm^2)	Tot. fibre area (mm^2)	Pith area (mm^2)	Xylem area (mm^2)	Phloem area (mm^2)
Viking	2.64 ±0.26	0.29 ±0.05	0.38±0.06	0.73 ±0.05	0.82±0.22
Hermes	1.95 ±0.11	0.23 ±0.02	0.28±0.05	0.49 ±0.06	0.61±0.09
E1747	1.54 ±0.19	0.13 ±0.01	0.15±0.02	0.43 ±0.01	0.52±0.03
H337	2.04 ±0.18	0.18 ±0.02	0.39±0.04	0.61 ±0.10	0.52±0.05
H341	1.95 ±0.57	0.18 ±0.01	0.23±0.02	0.54 ±0.02	0.38±0.04
H350	1.91 ±0.30	0.17 ±0.04	0.33±0.05	0.59 ±0.10	0.48±0.11
L260	1.62 ±0.14	0.10 ±0.01	0.27±0.02	0.46 ±0.05	0.44±0.04
L267	1.81 ±0.27	0.15 ±0.03	0.29±0.08	0.50 ±0.06	0.56±0.06
L271	1.55 ±0.17	0.12 ±0.00	0.31±0.03	0.51 ±0.01	0.45±0.01
D273	1.64 ±0.13	0.12 ±0.01	0.25±0.01	0.46 ±0.02	0.49±0.14
D311	1.93 ±0.12	0.18 ±0.03	0.26±0.03	0.53 ±0.04	0.65±0.04
D319	1.96 ±0.32	0.21 ±0.05	0.29±0.02	0.60 ±0.09	0.64±0.18
S268	1.53 ±0.13	0.15 ±0.02	0.31±0.05	0.39 ±0.01	0.42±0.05
T284	1.63 ±0.20	0.13 ±0.02	0.30±0.02	0.42 ±0.05	0.52±0.10

Seedling Growth chamber	Stem area (mm^2)	Tot. fibre area (mm^2)	Pith area (mm^2)	Xylem area (mm^2)	Phloem area (mm^2)
Viking	1.43 ±0.04	0.08 ±0.01	0.30 ±0.01	0.40 ±0.04	0.28 ±0.02
Hermes	1.57 ±0.15	0.13 ±0.03	0.37 ±0.05	0.37 ±0.05	0.30 ±0.02
E1747	1.05 ±0.10	0.06 ±0.01	0.20 ±0.01	0.26 ±0.02	0.25 ±0.08
H337	1.44 ±0.23	0.11 ±0.01	0.30 ±0.07	0.32 ±0.03	0.43 ±0.16
H341	1.43 ±0.05	0.09 ±0.01	0.19 ±0.05	0.31 ±0.07	0.25 ±0.07
H350	1.40 ±0.03	0.10 ±0.01	0.33 ±0.02	0.35 ±0.00	0.37 ±0.03
L260	1.36 ±0.07	0.07 ±0.01	0.29 ±0.03	0.33 ±0.02	0.33 ±0.03
L267	1.09 ±0.12	0.08 ±0.01	0.39 ±0.01	0.40 ±0.03	0.13 ±0.02
L271	1.20 ±0.07	0.06 ±0.00	0.27 ±0.02	0.30 ±0.01	0.17 ±0.01
D273	1.17 ±0.03	0.10 ±0.02	0.20 ±0.02	0.30 ±0.03	0.34 ±0.07
D311	1.12 ±0.07	0.15 ±0.01	0.20 ±0.04	0.31 ±0.01	0.31 ±0.01
D319	1.17 ±0.06	0.09 ±0.01	0.25 ±0.00	0.29 ±0.01	0.26 ±0.05
S268	1.75 ±0.22	0.11 ±0.02	0.47 ±0.07	0.43 ±0.06	0.45 ±0.09
T284	1.48 ±0.03	0.06 ±0.01	0.28 ±0.03	0.34 ±0.04	0.43 ±0.07

Table B.4.5. Mean of anatomical traits (Part B) for selected 14 genotypes at green capsule stage. (*Mean ± Standard Error)

GC Field	Stem area (mm^2)	Tot. fibre area (mm^2)	Pith area (mm^2)	Xylem area (mm^2)	Phloem area (mm^2)
Viking	5.96 ±0.63	0.64 ±0.06	1.04 ±0.04	2.85 ±0.37	1.36 ±0.18
Hermes	5.52 ±0.51	0.71 ±0.08	1.26 ±0.09	2.50 ±0.19	1.05 ±0.11
E1747	4.37 ±0.19	0.42 ±0.07	0.90 ±0.05	2.00 ±0.11	0.77 ±0.08
H337	3.20 ±0.43	0.55 ±0.21	0.78 ±0.09	1.49 ±0.19	0.70 ±0.19
H341	5.21 ±0.92	0.66 ±0.17	0.97 ±0.20	2.61 ±0.45	0.95 ±0.26
H350	4.52 ±0.22	0.40 ±0.06	0.75 ±0.08	2.22 ±0.15	0.98 ±0.04
L260	3.14 ±0.62	0.22 ±0.04	0.60 ±0.11	1.51 ±0.35	0.51 ±0.10
L267	2.05 ±0.35	0.16 ±0.02	0.42 ±0.05	0.90 ±0.20	0.35 ±0.05
L271	3.54 ±0.49	0.26 ±0.04	0.54 ±0.04	1.54 ±0.23	0.62 ±0.08
D273	3.19 ±0.26	0.27 ±0.05	0.83 ±0.12	1.44 ±0.21	0.41 ±0.06
D311	3.18 ±0.89	0.26 ±0.11	0.61 ±0.19	1.36 ±0.42	0.70 ±0.19
D319	4.27 ±1.04	0.52 ±0.22	0.67 ±0.15	1.91 ±0.47	0.98 ±0.33
S268	4.67 ±0.44	0.60 ±0.13	0.87 ±0.08	2.07 ±0.27	1.09 ±0.08
T284	4.31 ±0.34	0.39 ±0.05	0.83 ±0.06	2.21 ±0.24	0.66 ±0.06

GC Growth chamber	Stem area (mm^2)	Tot. fibre area (mm^2)	Pith area (mm^2)	Xylem area (mm^2)	Phloem area (mm^2)
Viking	5.75 ±0.61	0.62±0.12	1.11±0.08	2.36±0.28	1.50±0.18
Hermes	5.79 ±0.05	0.70±0.06	0.97±0.01	2.45±0.08	1.55±0.07
E1747	3.87 ±0.34	0.21±0.02	0.56±0.04	1.95±0.14	0.72±0.12
H337	3.72 ±0.05	0.35±0.01	0.77±0.09	1.54±0.05	0.89±0.03
H341	3.94 ±0.52	0.25±0.01	0.55±0.02	1.09±0.05	0.64±0.01
H350	4.28 ±0.44	0.45±0.05	0.69±0.06	1.97±0.27	1.00±0.08
L260	3.64 ±0.15	0.21±0.01	0.64±0.02	1.42±0.10	0.56±0.01
L267	2.81 ±0.17	0.23±0.04	0.34±0.03	1.48±0.06	0.63±0.13
L271	3.10 ±0.76	0.19±0.04	0.54±0.12	1.54±0.43	0.52±0.13
D273	2.86 ±0.13	0.14±0.01	0.48±0.03	1.37±0.02	0.39±0.09
D311	3.43 ±0.29	0.22±0.04	0.52±0.07	1.51±0.10	0.67±0.11
D319	3.35 ±0.21	0.27±0.03	0.68±0.03	1.39±0.11	0.67±0.08
S268	4.13 ±0.32	0.43±0.08	0.80±0.06	1.64±0.08	0.99±0.10
T284	4.10 ±0.05	0.24±0.02	0.69±0.09	1.93±0.14	0.73±0.23

Table B.4.6. Mean of anatomical traits (Part B) for selected 14 genotypes at physiological maturity stage. (*Mean ± Standard Error)

Maturity Field	Stem area (mm^2)	Tot. fibre area (mm^2)	Pith area (mm^2)	Xylem area (mm^2)	Phloem area (mm^2)
Viking	7.09 ±0.04	0.87 ±0.05	1.25 ±0.12	3.42 ±0.08	1.57 ±0.08
Hermes	6.64 ±0.87	0.80 ±0.09	1.33 ±0.06	2.84 ±0.51	1.64 ±0.20
E1747	4.71 ±0.59	0.50 ±0.08	0.92 ±0.02	2.17 ±0.30	0.83 ±0.21
H337	3.25 ±0.35	0.32 ±0.08	0.52 ±0.08	1.46 ±0.17	0.72 ±0.12
H341	2.41 ±0.16	0.24 ±0.01	0.52 ±0.04	0.96 ±0.12	0.52 ±0.02
H350	5.35 ±1.12	0.47 ±0.14	0.79 ±0.04	3.04 ±0.76	0.84 ±0.26
L260	3.12 ±0.46	0.22 ±0.05	0.54 ±0.09	1.35 ±0.25	0.66 ±0.14
L267	2.28 ±0.28	0.19 ±0.05	0.43 ±0.07	0.94 ±0.13	0.42 ±0.10
L271	3.35 ±0.32	0.19 ±0.01	0.68 ±0.07	1.45 ±0.25	0.66 ±0.03
D273	3.00 ±0.10	0.28 ±0.05	0.61 ±0.02	1.25 ±0.02	0.58 ±0.10
D311	4.63 ±0.58	0.57 ±0.10	0.94 ±0.24	2.00 ±0.33	0.81 ±0.04
D319	4.68 ±0.56	0.51 ±0.12	0.82 ±0.13	2.21 ±0.30	0.90 ±0.13
S268	3.89 ±0.40	0.45 ±0.09	0.74 ±0.10	1.64 ±0.19	0.75 ±0.14
T284	4.51 ±0.37	0.27 ±0.02	0.76 ±0.08	2.41 ±0.33	0.60 ±0.09

Maturity Growth chamber	Stem area (mm^2)	Tot. fibre area (mm^2)	Pith area (mm^2)	Xylem area (mm^2)	Phloem area (mm^2)
Viking	6.28 ±0.43	0.78 ±0.14	0.81 ±0.05	3.45 ±0.24	1.29 ±0.11
Hermes	6.86 ±0.62	1.19 ±0.30	0.74 ±0.04	3.44 ±0.28	1.92 ±0.35
E1747	4.70 ±0.21	0.25 ±0.02	0.54 ±0.03	2.61 ±0.22	0.86 ±0.06
H337	3.85 ±0.37	0.43 ±0.07	0.68 ±0.05	1.66 ±0.11	0.96 ±0.20
H341	3.23 ±0.23	0.27 ±0.01	0.42 ±0.05	1.78 ±0.09	0.55 ±0.08
H350	4.95 ±0.11	0.56 ±0.04	0.70 ±0.01	2.42 ±0.05	1.21 ±0.04
L260	3.44 ±0.17	0.24 ±0.04	0.72 ±0.00	1.63 ±0.09	0.57 ±0.06
L267	3.65 ±0.39	0.17 ±0.02	0.74 ±0.04	1.72 ±0.30	0.70 ±0.03
L271	3.85 ±0.11	0.18 ±0.01	0.70 ±0.07	2.22 ±0.16	0.54 ±0.01
D273	3.75 ±0.70	0.19 ±0.05	0.60 ±0.13	1.90 ±0.40	0.67 ±0.11
D311	4.04 ±0.69	0.20 ±0.04	0.68 ±0.09	2.16 ±0.41	0.53 ±0.09
D319	4.03 ±0.16	0.34 ±0.05	0.72 ±0.01	2.00 ±0.08	0.61 ±0.01
S268	4.77 ±0.48	0.57 ±0.14	0.77 ±0.07	2.06 ±0.26	1.34 ±0.13
T284	5.76 ±0.30	0.34 ±0.07	0.92 ±0.12	3.20 ±0.18	1.03 ±0.07

Table B.4.7. Mean of anatomical traits (Part C) for selected 14 genotypes at seedling stage. (*Mean ± Standard Error)

Seedling Field	Fibre: Stem	Fibre: Phloem	Fibre: Xylem	Xylem: Stem	Pith: Stem
Viking	0.11 ±0.01	0.37 ±0.05	0.40 ±0.05	0.28 ±0.02	0.15 ±0.03
Hermes	0.12 ±0.02	0.39 ±0.05	0.50 ±0.09	0.25 ±0.02	0.15 ±0.03
E1747	0.08 ±0.00	0.24 ±0.00	0.30 ±0.02	0.28 ±0.01	0.10 ±0.01
H337	0.09 ±0.01	0.35 ±0.03	0.31 ±0.03	0.29 ±0.03	0.19 ±0.02
H341	0.09 ±0.00	0.50 ±0.06	0.34 ±0.01	0.28 ±0.00	0.12 ±0.01
H350	0.09 ±0.01	0.36 ±0.01	0.28 ±0.02	0.31 ±0.01	0.18 ±0.02
L260	0.06 ±0.01	0.24 ±0.03	0.23 ±0.02	0.28 ±0.01	0.17 ±0.01
L267	0.08 ±0.01	0.26 ±0.03	0.28 ±0.03	0.28 ±0.01	0.15 ±0.02
L271	0.08 ±0.01	0.27 ±0.02	0.23 ±0.01	0.34 ±0.03	0.21 ±0.03
D273	0.08 ±0.00	0.29 ±0.06	0.28 ±0.01	0.28 ±0.03	0.15 ±0.02
D311	0.09 ±0.01	0.27 ±0.03	0.34 ±0.06	0.27 ±0.01	0.13 ±0.01
D319	0.10 ±0.01	0.33 ±0.03	0.34 ±0.04	0.31 ±0.02	0.15 ±0.02
S268	0.10 ±0.01	0.37 ±0.04	0.39 ±0.05	0.26 ±0.02	0.20 ±0.01
T284	0.08 ±0.00	0.26 ±0.03	0.32 ±0.02	0.26 ±0.02	0.19 ±0.01

Seedling Growth chamber	Fibre: Stem	Fibre: Phloem	Fibre: Xylem	Xylem: Stem	Pith: Stem
Viking	0.06 ±0.01	0.31 ±0.07	0.21 ±0.02	0.28 ±0.02	0.21 ±0.01
Hermes	0.08 ±0.01	0.43 ±0.11	0.33 ±0.03	0.24 ±0.01	0.23 ±0.02
E1747	0.06 ±0.01	0.30 ±0.13	0.21 ±0.03	0.25 ±0.03	0.19 ±0.02
H337	0.08 ±0.01	0.39 ±0.20	0.34 ±0.03	0.23 ±0.04	0.20 ±0.02
H341	0.06 ±0.00	0.39 ±0.10	0.32 ±0.10	0.22 ±0.05	0.14 ±0.04
H350	0.07 ±0.01	0.29 ±0.04	0.29 ±0.02	0.25 ±0.01	0.24 ±0.02
L260	0.05 ±0.00	0.21 ±0.04	0.21 ±0.01	0.24 ±0.01	0.21 ±0.01
L267	0.07 ±0.01	0.65 ±0.16	0.20 ±0.05	0.38 ±0.06	0.37 ±0.04
L271	0.05 ±0.00	0.36 ±0.02	0.20 ±0.01	0.25 ±0.02	0.22 ±0.00
D273	0.08 ±0.02	0.31 ±0.09	0.32 ±0.06	0.26 ±0.03	0.17 ±0.02
D311	0.13 ±0.02	0.46 ±0.04	0.47 ±0.05	0.28 ±0.01	0.18 ±0.03
D319	0.08 ±0.01	0.37 ±0.10	0.31 ±0.04	0.25 ±0.01	0.22 ±0.02
S268	0.07 ±0.01	0.27 ±0.05	0.27 ±0.02	0.25 ±0.02	0.27 ±0.02
T284	0.04 ±0.01	0.15 ±0.03	0.18 ±0.03	0.23 ±0.03	0.19 ±0.02

Table B.4.8. Mean of anatomical traits (Part C) for selected 14 genotypes at the green capsule stage. (*Mean ± Standard Error)

GC Field	Fibre: Stem	Fibre: Phloem	Fibre: Xylem	Xylem: Stem	Pith: Stem
Viking	0.11 ±0.00	0.48 ±0.04	0.23 ±0.01	0.48 ±0.01	0.18 ±0.01
Hermes	0.13 ±0.01	0.67 ±0.03	0.28 ±0.01	0.45 ±0.01	0.23 ±0.01
E1747	0.10 ±0.01	0.55 ±0.06	0.21 ±0.03	0.46 ±0.01	0.21 ±0.02
H337	0.16 ±0.05	0.73 ±0.11	0.35 ±0.12	0.47 ±0.01	0.25 ±0.01
H341	0.12 ±0.02	0.70 ±0.09	0.25 ±0.03	0.50 ±0.01	0.19 ±0.01
H350	0.09 ±0.01	0.41 ±0.07	0.18 ±0.03	0.49 ±0.01	0.17 ±0.02
L260	0.07 ±0.00	0.46 ±0.08	0.15 ±0.01	0.48 ±0.02	0.19 ±0.02
L267	0.08 ±0.00	0.47 ±0.01	0.19 ±0.02	0.43 ±0.02	0.21 ±0.01
L271	0.07 ±0.00	0.41 ±0.03	0.17 ±0.01	0.43 ±0.01	0.16 ±0.01
D273	0.08 ±0.01	0.71 ±0.18	0.19 ±0.02	0.45 ±0.03	0.26 ±0.03
D311	0.08 ±0.01	0.35 ±0.05	0.18 ±0.03	0.43 ±0.02	0.19 ±0.04
D319	0.11 ±0.02	0.50 ±0.05	0.25 ±0.05	0.45 ±0.01	0.16 ±0.02
S268	0.13 ±0.02	0.54 ±0.10	0.28 ±0.03	0.44 ±0.02	0.19 ±0.01
T284	0.09 ±0.01	0.59 ±0.06	0.18 ±0.01	0.51 ±0.02	0.19 ±0.02

GC Growth chamber	Fibre: Stem	Fibre: Phloem	Fibre: Xylem	Xylem: Stem	Pith: Stem
Viking	0.11 ±0.01	0.41 ±0.06	0.26 ±0.03	0.41 ±0.01	0.20 ±0.01
Hermes	0.12 ±0.01	0.46 ±0.04	0.29 ±0.03	0.42 ±0.02	0.17 ±0.00
E1747	0.06 ±0.00	0.31 ±0.02	0.11 ±0.00	0.51 ±0.01	0.14 ±0.00
H337	0.09 ±0.00	0.39 ±0.02	0.23 ±0.01	0.41 ±0.01	0.21 ±0.02
H341	0.07 ±0.01	0.39 ±0.01	0.23 ±0.01	0.29 ±0.04	0.15 ±0.02
H350	0.11 ±0.01	0.45 ±0.02	0.23 ±0.02	0.46 ±0.02	0.16 ±0.00
L260	0.06 ±0.00	0.38 ±0.03	0.15 ±0.01	0.39 ±0.01	0.18 ±0.01
L267	0.08 ±0.01	0.37 ±0.01	0.15 ±0.02	0.53 ±0.01	0.12 ±0.01
L271	0.06 ±0.00	0.39 ±0.04	0.13 ±0.01	0.49 ±0.03	0.18 ±0.01
D273	0.05 ±0.00	0.39 ±0.08	0.10 ±0.01	0.48 ±0.02	0.17 ±0.01
D311	0.06 ±0.01	0.34 ±0.07	0.14 ±0.02	0.44 ±0.02	0.15 ±0.01
D319	0.08 ±0.01	0.41 ±0.04	0.19 ±0.01	0.42 ±0.01	0.20 ±0.00
S268	0.10 ±0.01	0.44 ±0.04	0.26 ±0.03	0.40 ±0.01	0.20 ±0.01
T284	0.06 ±0.01	0.47 ±0.21	0.13 ±0.02	0.47 ±0.03	0.17 ±0.02

Table B.4.9. Mean of anatomical traits (Part C) for selected 14 genotypes at physiological maturity stage. (*Mean ± Standard Error)

Maturity Field	Fibre: Stem	Fibre: Phloem	Fibre: Xylem	Xylem: Stem	Pith: Stem
Viking	0.12 ±0.01	0.56 ±0.05	0.25 ±0.01	0.48 ±0.01	0.18 ±0.02
Hermes	0.12 ±0.00	0.49 ±0.04	0.29 ±0.02	0.42 ±0.02	0.21 ±0.02
E1747	0.10 ±0.01	0.62 ±0.04	0.23 ±0.01	0.46 ±0.01	0.20 ±0.02
H337	0.10 ±0.01	0.44 ±0.03	0.22 ±0.03	0.45 ±0.01	0.16 ±0.02
H341	0.10 ±0.00	0.46 ±0.03	0.25 ±0.02	0.40 ±0.02	0.22 ±0.01
H350	0.08 ±0.01	0.58 ±0.04	0.15 ±0.01	0.56 ±0.03	0.16 ±0.04
L260	0.07 ±0.01	0.33 ±0.05	0.16 ±0.02	0.43 ±0.04	0.17 ±0.01
L267	0.08 ±0.01	0.46 ±0.03	0.21 ±0.04	0.41 ±0.03	0.19 ±0.01
L271	0.06 ±0.01	0.29 ±0.01	0.14 ±0.02	0.43 ±0.04	0.21 ±0.03
D273	0.09 ±0.01	0.51 ±0.11	0.23 ±0.03	0.42 ±0.01	0.20 ±0.01
D311	0.12 ±0.01	0.71 ±0.14	0.29 ±0.02	0.43 ±0.03	0.20 ±0.03
D319	0.11 ±0.01	0.55 ±0.05	0.23 ±0.03	0.47 ±0.01	0.17 ±0.01
S268	0.11 ±0.01	0.60 ±0.03	0.27 ±0.02	0.42 ±0.01	0.19 ±0.02
T284	0.06 ±0.01	0.47 ±0.08	0.12 ±0.02	0.53 ±0.03	0.17 ±0.01

Maturity Growth chamber	Fibre: Stem	Fibre: Phloem	Fibre: Xylem	Xylem: Stem	Pith: Stem
Viking	0.12 ±0.02	0.60 ±0.08	0.23 ±0.03	0.55 ±0.01	0.13 ±0.00
Hermes	0.17 ±0.03	0.61 ±0.04	0.34 ±0.06	0.50 ±0.01	0.11 ±0.02
E1747	0.05 ±0.00	0.29 ±0.03	0.10 ±0.01	0.55 ±0.03	0.12 ±0.01
H337	0.11 ±0.01	0.45 ±0.03	0.25 ±0.03	0.43 ±0.02	0.18 ±0.01
H341	0.08 ±0.00	0.51 ±0.07	0.15 ±0.00	0.55 ±0.02	0.13 ±0.01
H350	0.11 ±0.01	0.46 ±0.03	0.23 ±0.02	0.49 ±0.00	0.14 ±0.00
L260	0.07 ±0.01	0.44 ±0.08	0.15 ±0.03	0.48 ±0.01	0.21 ±0.01
L267	0.05 ±0.01	0.25 ±0.04	0.10 ±0.01	0.46 ±0.04	0.20 ±0.01
L271	0.05 ±0.00	0.33 ±0.02	0.08 ±0.01	0.57 ±0.03	0.18 ±0.02
D273	0.05 ±0.01	0.27 ±0.04	0.10 ±0.01	0.50 ±0.02	0.16 ±0.01
D311	0.05 ±0.00	0.37 ±0.02	0.09 ±0.00	0.53 ±0.01	0.17 ±0.01
D319	0.08 ±0.01	0.55 ±0.08	0.17 ±0.02	0.50 ±0.01	0.18 ±0.01
S268	0.12 ±0.02	0.41 ±0.07	0.27 ±0.04	0.43 ±0.02	0.16 ±0.01
T284	0.06 ±0.01	0.33 ±0.05	0.11 ±0.02	0.56 ±0.03	0.16 ±0.01

Appendix C

Table C. 1. List of EST-SSRs, number of repeats and primer design identified from Day et al. (2005) 927 EST GenBank collection used in the molecular marker study.

Sl.No	Marker name	Sequence ID	Repeat	Left Sequence	Right Sequence	Product Size	Tm
1	CV478070	>gi|53702846|gb|CV478070.1|CV478070	(GAT)4	ATATGATCCAGGGTCCTCTT	TCCAGAGGTTGAAGAAGGTA	306	55.0
2	CV478078	>gi|53702854|gb|CV478078.1|CV478078	(TCA)4	ACCGCTAATTGCATAGACAT	CACCACAGACAAACAACAAG	229	55.0
3	CV478095	>gi|53702871|gb|CV478095.1|CV478095	(CCA)4	ACAGGTACCAATCATCAAGC	GTTCTCCGTCATCAACTCAT	383	55.0
4	CV478099	>gi|53702875|gb|CV478099.1|CV478099	(AGAA)3	ACATGTCGGAGATGAGCTT	AGAACTAGCCACAGCAACAT	257	55.3
5	CV478105	>gi|53702881|gb|CV478105.1|CV478105	(TGA)4	GTTTGACCTGCAATAAGAGG	CCCACAAATTTCATAATCGT	360	55.0
6	CV478110	>gi|53702886|gb|CV478110.1|CV478110	(AGA)4	TCCGTATTGAAGAAGAGGAA	TGACATAGAAACCACCCTTC	202	55.0
7	CV478111	>gi|53702887|gb|CV478111.1|CV478111	(ATT)5	ATCATCTTCACATCGGTGAT	GAACAAAGAAACTGCAAACC	293	55.1
8	CV478111	>gi|53702887|gb|CV478111.1|CV478111	(GAGT)3	ATCATCTTCACATCGGTGAT	GAACAAAGAAACTGCAAACC	293	55.1
9	CV478113	>gi|53702889|gb|CV478113.1|CV478113	(AGAA)3	CGGTGTTTGATGTTTGGTAT	CACGGAAATCAAAGAAGAAC	151	55.4
10	CV478124	>gi|53702900|gb|CV478124.1|CV478124	(GAG)4	AGTATTATCATCCCGGAGGT	CACCCTCTCTGCAATTCTAC	347	55.0
11	CV478135	>gi|53702911|gb|CV478135.1|CV478135	(CCA)5	TGGAAGAAGCCACAAATACT	AGTGCATCTGGTGGTGGT	307	56.1
12	CV478167	>gi|53702943|gb|CV478167.1|CV478167	(CAA)4	ACAGAAAGTCGTCAAAGGAA	CATCTCCAAATTGAATGGTC	328	55.2
13	CV478177	>gi|53702953|gb|CV478177.1|CV478177	(CTTC)3	TGCTATCACTTGTCACATTCA	GACTTACTGCAGACAGCACA	348	55.1
14	CV478177	>gi|53702953|gb|CV478177.1|CV478177	(ACT)7	TTTCTCCAAGCTTCAACTGT	ATGGCATATAGAGCACTCGT	203	55.0
15	CV478194	>gi|53702970|gb|CV478194.1|CV478194	(GAT)4	CAAGAAGAAGATCGAGGATG	AAACTGATACAATGGCCAAC	285	55.0
16	CV478207	>gi|53702983|gb|CV478207.1|CV478207	(CCA)4	ATAACAACCTACGAAGGCAA	GTTGGTGAGATCAAGGGTTA	246	55.0
17	CV478207	>gi|53702983|gb|CV478207.1|CV478207	(CCG)4	ATAACAACCTACGAAGGCAA	CAATGGCAGCAGTCATAGTA	382	55.0
18	CV478207	>gi|53702983|gb|CV478207.1|CV478207	(ATA)6	TAACCCTTGATCTCACCAAC	GGTGGAGAAAGTAGTGCAAG	305	55.0
19	CV478210	>gi|53702986|gb|CV478210.1|CV478210	(CTT)10	ATATCCATTTGTGAGGGATG	TCTCAGAGTTCATCAAACGA	266	54.5
20	CV478211	>gi|53702987|gb|CV478211.1|CV478211	(GAT)5	CAAGCAACAATCAAGACGTA	TCGACTGACCTCGTTAATTT	350	55.0
21	CV478211	>gi|53702987|gb|CV478211.1|CV478211	(CTC)4	ATGATGATGATGATGGGTTT	TCGACTGACCTCGTTAATTT	129	55.0

Sl.No	Marker name	Sequence ID	Repeat	Left Sequence	Right Sequence	Product Size	Tm
22	CV478211	>gi|53702987|gb|CV478211.1|CV478211	(AT)6	GAAGATATCAGTCGCAAAGG	TCTCCATCTCTGGAAACCTA	387	54.9
23	CV478217	>gi|53702993|gb|CV478217.1|CV478217	(ATT)4	ATAAGTGGTCGGCTAACAAA	TCTGTTTACCAATCTTTGCC	342	55.2
24	CV478219	>gi|53702995|gb|CV478219.1|CV478219	(CGATT)3	GTCGTCTTCGACATCTTCAC	AACGCCTTGAGCTTATTAAA	314	55.2
25	CV478227	>gi|53703003|gb|CV478227.1|CV478227	(AGAA)7	CGGTGTTTGATGTTTGGTAT	AATTGACTGACACGGAAATC	173	55.4
26	CV478231	>gi|53703007|gb|CV478231.1|CV478231	(TCC)4	GTACAGGGAGAGACCCAGA	TGAGTCAACTCGGAGCTTAT	372	55.0
27	CV478237	>gi|53703013|gb|CV478237.1|CV478237	(GCA)4	GACGAACTATTGGCTCTCAG	GGTTGTTGATGGTATGCTCT	265	55.1
28	CV478252	>gi|53703028|gb|CV478252.1|CV478252	(CAA)7	AAAGAAACTTGTGCTTCAGTG	TTCTGCTTCAAGTTGTTGTG	388	54.9
29	CV478252	>gi|53703028|gb|CV478252.1|CV478252	(TACG)3	GCAACAACAACAACAACAAC	GTGGCTCTGGTTTAATATGG	314	54.9
30	CV478252	>gi|53703028|gb|CV478252.1|CV478252	(CAA)6	GCAACAACAACAACAACAAC	GTGGCTCTGGTTTAATATGG	314	54.9
31	CV478252	>gi|53703028|gb|CV478252.1|CV478252	(TCA)4	CACAACAACTTGAAGCAGAA	ACGAACATAGCAGCAGAAAG	307	55.4
32	CV478263	>gi|53703039|gb|CV478263.1|CV478263	(GGC)5	TCAACTTCCACTCTGCTACA	CAGACACATAACCAACATGC	163	54.6
33	CV478265	>gi|53703041|gb|CV478265.1|CV478265	(TCC)4	AGCCAGAAGTGATGTTCATT	CTCAGTCAATCCAATCCAAT	351	54.8
34	CV478265	>gi|53703041|gb|CV478265.1|CV478265	(TGAT)3	CAGGACTTAATCCTCCTCCT	TGCAATGACCATTTACAGAA	382	55.0
35	CV478275	>gi|53703051|gb|CV478275.1|CV478275	(TC)5	TACTACTGGTGGATATGGGC	ATTAAACCACCAACGTCAAT	174	54.8
36	CV478277	>gi|53703053|gb|CV478277.1|CV478277	(TTC)6	TTATCGGATTCTCCATTGTC	CTGAAGAGCAGAGTTCCATC	334	55.1
37	CV478279	>gi|53703055|gb|CV478279.1|CV478279	(GTCAG)3	TACGAGAAGGCTGTCAGAAT	TGGGTTCTGTAGCTTGTTCT	379	55.1
38	CV478280	>gi|53703056|gb|CV478280.1|CV478280	(GAA)7	ACCCTAAGTCCTCGTCTCTC	TCCTCAGATTCAGAACCATC	274	55.0
39	CV478291	>gi|53703067|gb|CV478291.1|CV478291	(GAA)7	ACTGAATACCGAGAACGAAA	CGTCTCCACCTATCATCATC	242	55.2
40	CV478303	>gi|53703079|gb|CV478303.1|CV478303	(GATGT)3	TTTGCGACTTCTAGTCTTCC	TGTTGTTTCCCAAGCTTAAT	202	54.9
41	CV478306	>gi|53703082|gb|CV478306.1|CV478306	(TAG)6	AGAAGGGATTGGAAGTGAAT	CCATGGAAGTAATGAGGAAA	182	55.2
42	CV478315	>gi|53703091|gb|CV478315.1|CV478315	(AAG)5	ACAAGCTGTTCCTTATTGGA	AGCAGCTCATAATGTTGGTT	294	55.0
43	CV478319	>gi|53703095|gb|CV478319.1|CV478319	(ATG)5	GCAGAACTTTAGGTCCATTG	ATCGGAAATTCACTTGTCTG	190	55.1
44	CV478331	>gi|53703107|gb|CV478331.1|CV478331	(GTG)4	ATGAAGAATGTGACCGTTTC	TCTCTTCTTTCCAGAGGTCA	359	55.0
45	CV478337	>gi|53703113|gb|CV478337.1|CV478337	(CCA)4	ATGGATTGGAAGGTCCTATT	TGAAAGGGATCGTCTCTCTA	379	55.0
46	CV478340	>gi|53703116|gb|CV478340.1|CV478340	(GCA)4	AAACAGACGATGATTCTGCT	CAACAACGACAGCTTAACAA	271	55.0
47	CV478346	>gi|53703122|gb|CV478346.1|CV478346	(GT)8	CAGTTCTCATTGGTGGATCT	TTGGAACTTTCCACTAAAGG	343	54.8

Sl.No	Marker name	Sequence ID	Repeat	Left Sequence	Right Sequence	Product Size	Tm
48	CV478350	>gi|53703126|gb|CV478350.1|CV478350	(CCT)4	CAGCTCAATTGCTTCTTCTT	AGAGTAACCTATGCCAACGA	359	55.0
49	CV478350	>gi|53703126|gb|CV478350.1|CV478350	(TA)5	TCGTTGGCATAGGTTACTCT	TTTACACAAACATTCAGTTACA	264	53.1
50	CV478367	>gi|53703143|gb|CV478367.1|CV478367	(TCT)4	TGAAATTGGAACACACAGAA	TTATTTCATTCACCATACATAAA	203	53.5
51	CV478381	>gi|53703157|gb|CV478381.1|CV478381	(TACAA)3	CTTCTTGCTAGAGGCTGTGT	CCGTCAATTTACGATACAGA	329	54.4
52	CV478384	>gi|53703160|gb|CV478384.1|CV478384	(AAAAC)3	GACATGTACGAGTAGCCCTC	ACCCTCAGAATGGATAGGTT	393	54.9
53	CV478385	>gi|53703161|gb|CV478385.1|CV478385	(AAT)5	TCCGTTTCACCTTACAGAGT	CTGCTTTGTTGGTTGTGTAA	361	54.9
54	CV478385	>gi|53703161|gb|CV478385.1|CV478385	(ATAC)5	TAAGTCGAGGAAGAAAGCAG	GGAGCTGAATGAATGAAAGA	203	55.2
55	CV478411	>gi|53703187|gb|CV478411.1|CV478411	(GAA)5	AATCTCCTCCACCATCGG	AAGCTCATGAGAGGATGATG	223	56.8
56	CV478416	>gi|53703192|gb|CV478416.1|CV478416	(AGAA)3	CGGTGTTTGATGTTTGGTAT	CACGGAAATCAAAGAAGAAC	151	55.4
57	CV478441	>gi|53703217|gb|CV478441.1|CV478441	(GAA)5	AGAATGGCTTCACGAAGAT	GAGATTAGCATGAGTTTGCC	268	54.9
58	CV478452	>gi|53703228|gb|CV478452.1|CV478452	(TCT)4	CTAATGGCGCAAACTAAATC	GGAGGGAGAAAGTTGAAAGT	163	55.1
59	CV478455	>gi|53703231|gb|CV478455.1|CV478455	(ACCG)3	AGTGAATCGTCGTTGGATAC	TCAATTCCATCCACCTAAAC	176	55.0
60	CV478464	>gi|53703240|gb|CV478464.1|CV478464	(CCAG)4	GGGCAGTCCTGATCATAATA	GATTAAACAAAGTGATACCAACC	371	54.8
61	CV478470	>gi|53703246|gb|CV478470.1|CV478470	(GCA)4	CTTCATTCAGATCCATACCC	TGCTGCTTCTGATACTGTTG	296	54.8
62	CV478470	>gi|53703246|gb|CV478470.1|CV478470	(GAT)4	CTTCATTCAGATCCATACCC	TGCTGCTTCTGATACTGTTG	296	54.8
63	CV478476	>gi|53703252|gb|CV478476.1|CV478476	(ATT)4	GTAAGCACCTGAGCGTAGTT	GAACAAAGAAACTGCAAACC	351	54.9
64	CV478476	>gi|53703252|gb|CV478476.1|CV478476	(GAGT)3	GTAAGCACCTGAGCGTAGTT	GAACAAAGAAACTGCAAACC	351	54.9
65	CV478477	>gi|53703253|gb|CV478477.1|CV478477	(AGC)4	TCAAATCAACACCAAAGTGA	GCAATAGCTCCACCAACTAC	163	55.0
66	CV478478	>gi|53703254|gb|CV478478.1|CV478478	(TCTT)4	GGGTTTGTAGTAGTGCCTTG	TGGCGCATTATCTTATCTTT	249	55.0
67	CV478481	>gi|53703257|gb|CV478481.1|CV478481	(ATGGG)3	GACCAGCAGATCTACAATCC	TATCTCGCACTTGAATTCCT	111	54.9
68	CV478481	>gi|53703257|gb|CV478481.1|CV478481	(GATC)4	AGGAATTCAAGTGCGAGATA	ACCAATTGAGTAACTCGGTG	357	55.1
69	CV478498	>gi|53703274|gb|CV478498.1|CV478498	(TAC)4	CTCTCGCTCACTGAAGCTAT	AAATTTGCAGTGATTCCAGT	356	54.9
70	CV478501	>gi|53703277|gb|CV478501.1|CV478501	(AAC)4	GAGTCGATGAAATGAGAAGC	GACTGGTTTGGACAAGTGAT	237	54.9
71	CV478502	>gi|53703278|gb|CV478502.1|CV478502	(ATCA)4	TTTCCTTAACTCCGATAAA	TTCATCACTTCAGGCTCTCT	295	52.6
72	CV478506	>gi|53703282|gb|CV478506.1|CV478506	(TA)13	CCAACTACGTACTCTCCAGC	ACGAAATAGGATGGAGGAAG	156	55.4
73	CV478510	>gi|53703286|gb|CV478510.1|CV478510	(AAG)4	CATCTTCTTGAGTTTCTGGG	ATTAAGGCAACCACAAAGAA	290	54.9

Sl.No	Marker name	Sequence ID	Repeat	Left Sequence	Right Sequence	Product Size	Tm
74	CV478519	>gi|53703295|gb|CV478519.1|CV478519	(TCT)4	GCTCTAGCGTCAGCTCTTAG	TGATGAAACAGCATAGGAGTT	348	55.0
75	CV478533	>gi|53703309|gb|CV478533.1|CV478533	(CCAT)3	GTCATGATGGCCAAGTAAGT	GGAACCTTCAATTCATTTCA	256	55.1
76	CV478541	>gi|53703317|gb|CV478541.1|CV478541	(GCT)4	AAACTAAGATCTCCGCTTGA	TCACTTGTTGCTCTTGAGTG	187	54.7
77	CV478560	>gi|53703336|gb|CV478560.1|CV478560	(CTT)4	CTCAAAGATAAACCAGCACC	GAAACTAAGTGCACGACTCC	126	55.0
78	CV478569	>gi|53703345|gb|CV478569.1|CV478569	(CAG)4	TGTCTGGTTATTGTTCGTGA	CGCTATGCTTCTCTGTTTCT	266	55.0
79	CV478574	>gi|53703350|gb|CV478574.1|CV478574	(CAA)4	GCTGAACTCCCAGTACAAAG	CTGGGAATGGTAGTAGTTCG	253	54.9
80	CV478607	>gi|53703383|gb|CV478607.1|CV478607	(CTC)4	TTTGGCAACTCTCTGAATTT	ATCAACTCGAAAGCTTGGTA	347	55.1
81	CV478607	>gi|53703383|gb|CV478607.1|CV478607	(GCC)4	TTTGGCAACTCTCTGAATTT	ATCAACTCGAAAGCTTGGTA	347	55.1
82	CV478609	>gi|53703385|gb|CV478609.1|CV478609	(TA)13	TCTCCAGCATTCTATCGTCT	GAACAAATTCTCAACCGTTC	215	54.9
83	CV478611	>gi|53703387|gb|CV478611.1|CV478611	(CCT)8	CTCCTTCAACCCTCTTAACC	TAAGGTTGTCTAAGAAGGCG	326	55.2
84	CV478613	>gi|53703389|gb|CV478613.1|CV478613	(GCA)4	CGCCTACAAGAGAACACAGT	GTGTTGGTGGCTGAGATAGT	278	55.3
85	CV478613	>gi|53703389|gb|CV478613.1|CV478613	(CAT)5	GAGCTCTCCAAGATCAACAG	AAGTTCGACACTCCGAAATA	386	55.0
86	CV478623	>gi|53703399|gb|CV478623.1|CV478623	(ATT)4	GTAAGCACCTGAGCGTAGTT	GAACAAAGAAACTGCAAACC	350	54.9
87	CV478623	>gi|53703399|gb|CV478623.1|CV478623	(GAGT)3	GTAAGCACCTGAGCGTAGTT	GAACAAAGAAACTGCAAACC	350	54.9
88	CV478626	>gi|53703402|gb|CV478626.1|CV478626	(GCC)4	CCGTCTTATTCCTCTTCCTT	TCCGGAACATACTCCATAAC	399	55.1
89	CV478634	>gi|53703410|gb|CV478634.1|CV478634	(AGG)8	CTGAGAATGAGAAGCTGTCC	TTATGAACCAAGTGAGTCCC	294	55.0
90	CV478636	>gi|53703412|gb|CV478636.1|CV478636	(AGAC)5	ACTTGGATGTGCATCTTTCT	CAATCAACAATCCATACCCT	198	54.8
91	CV478639	>gi|53703415|gb|CV478639.1|CV478639	(TGGTT)3	GATGACGTGGGTTACACATC	GTAGCACCGGTGAAGTAGAG	344	55.6
92	CV478643	>gi|53703419|gb|CV478643.1|CV478643	(AGC)4	GGTGATGACTTGGTTGTTCT	TACAGACAATATGTCGAGCG	171	54.9
93	CV478655	>gi|53703431|gb|CV478655.1|CV478655	(TCC)4	ATGGAGTAGCAGCAGAAGAA	GAGGAGGGAGAAGAGTTTGT	123	55.1
94	CV478655	>gi|53703431|gb|CV478655.1|CV478655	(CTC)6	ACAAACTCTTCTCCCTCCTC	CTTCTTAGCATTCTGGTGCT	340	54.9
95	CV478671	>gi|53703447|gb|CV478671.1|CV478671	(CCG)4	AATCACAATGGAAAGAGACG	AATTGGAAGTGAGGATTGTG	132	55.1
96	CV478678	>gi|53703454|gb|CV478678.1|CV478678	(GCA)4	AAGCTAAGGGTATGAAGCCT	ACTGAGGCTACCTGCTACAA	285	55.1
97	CV478680	>gi|53703456|gb|CV478680.1|CV478680	(TGCT)3	AACCAAATGGATTTGATACG	TAAAGAAGCTACCAGTTGGG	371	54.9
98	CV478683	>gi|53703459|gb|CV478683.1|CV478683	(GAGT)3	GGCAAAGATTGGTAAACAGA	GAACAAAGAAACTGCAAACC	121	55.2
99	CV478684	>gi|53703460|gb|CV478684.1|CV478684	(TTC)4	CAACACTTTCGTCGGTAAAT	AGCTCGATGAGATCGTAGAA	362	55.3

Sl.No	Marker name	Sequence ID	Repeat	Left Sequence	Right Sequence	Product Size	Tm
100	CV478684	>gi|53703460|gb|CV478684.1|CV478684	(GGT)4	CAACACTTTCGTCGGTAAAT	AGCTCGATGAGATCGTAGAA	362	55.3
101	CV478701	>gi|53703477|gb|CV478701.1|CV478701	(TTCT)3	GGAAGGGAGAAGGGTATCTA	TGCTGATGCTCTTTAGTCAG	153	54.6
102	CV478705	>gi|53703481|gb|CV478705.1|CV478705	(CCT)4	TGTCCCTACGATTTGGTTAC	GTAATTAACAGCCCAGATGC	243	55.0
103	CV478713	>gi|53703489|gb|CV478713.1|CV478713	(GAG)5	ACCAACATCATTTACAAGCC	TACTGTGATCCACAACATGG	277	55.1
104	CV478717	>gi|53703493|gb|CV478717.1|CV478717	(TCT)4	TGGCACTCTAAACAGGTTCT	AAAGCAACCAAACCAAGTT	260	54.9
105	CV478718	>gi|53703494|gb|CV478718.1|CV478718	(GAT)5	ACAGGAATCGACTGATATGG	TGCATATGCTCATAAACAGC	394	55.0
106	CV478726	>gi|53703502|gb|CV478726.1|CV478726	(TC)16	TATTCCACCCGAATATCAAC	AAGCACAACAAAGGACAAAT	347	54.9
107	CV478734	>gi|53703510|gb|CV478734.1|CV478734	(ATC)4	GTACCAACCATTATCGTCGT	ACTCCTGGTGCGTTCTATTA	384	55.0
108	CV478735	>gi|53703511|gb|CV478735.1|CV478735	(AGC)5	CAGATGAATTCCCACATCTT	CTTCTTGTCCGCTCAAACT	217	55.3
109	CV478761	>gi|53703537|gb|CV478761.1|CV478761	(CAG)4	GCTTCAGATTACAGCCAATC	AATCACTGAAACTGATTCTGG	344	54.7
110	CV478764	>gi|53703540|gb|CV478764.1|CV478764	(GAA)4	ACACCAGAGACATGGAGAAG	TGATCTCACCACATGCTCTA	271	55.1
111	CV478768	>gi|53703544|gb|CV478768.1|CV478768	(CAT)4	GATCAGCCTAGCTGTCTCTC	CTGAACCAACATCAACTCCT	331	54.7
112	CV478786	>gi|53703562|gb|CV478786.1|CV478786	(CCA)6	TGGAAGAAGCCACAAATACT	AAAGGGAGGGTAGTAAATCC	383	54.5
113	CV478788	>gi|53703564|gb|CV478788.1|CV478788	(TAAT)4	AAGGAGGAGAAATCTGAAGG	AACCATTAAACCTCGCATAA	147	55.0
114	CV478790	>gi|53703566|gb|CV478790.1|CV478790	(CAG)6	TAACATCGGTGTGGTATTCA	ATTCTGGAGGCATATTGTTG	383	55.0
115	CV478808	>gi|53703584|gb|CV478808.1|CV478808	(GAT)5	CAAGAAGAAGATCGAGGATG	AGAAATTTAGTCCGCCTCTT	239	54.9
116	CV478819	>gi|53703595|gb|CV478819.1|CV478819	(TAA)5	CTGCCGCTAACAACTACTCT	GAACTCGGAATAAACATCCA	296	55.1
117	CV478824	>gi|53703600|gb|CV478824.1|CV478824	(CTAG)5	CATTGGTTACACCCTTGTCT	GGTTGATGGTATTGGGTAGA	254	54.9
118	CV478825	>gi|53703601|gb|CV478825.1|CV478825	(GGAT)4	AACCTTCTCCTGTGTTCTCA	TATTCTTTCTTCCGTCATGG	381	55.0
119	CV478830	>gi|53703606|gb|CV478830.1|CV478830	(ATT)4	GTAAGCACCTGAGCGTAGTT	GAACAAAGAAACTGCAAACC	350	54.9
120	CV478830	>gi|53703606|gb|CV478830.1|CV478830	(GAGT)3	GTAAGCACCTGAGCGTAGTT	GAACAAAGAAACTGCAAACC	350	54.9
121	CV478831	>gi|53703607|gb|CV478831.1|CV478831	(CAG)7	GGTAATTTGTCTACTGTGCCA	TCTGCAGATCGTATTCATTG	133	55.1
122	CV478838	>gi|53703614|gb|CV478838.1|CV478838	(AGC)5	CAATTTCTTCTCTTCCAACG	GAACATTGCCTTGATTGACT	399	55.1
123	CV478840	>gi|53703616|gb|CV478840.1|CV478840	(CT)7	CCCTTCACCTCTCTCTCTCT	CGTAGGTTTAATCCTTGTCG	340	55.1
124	CV478840	>gi|53703616|gb|CV478840.1|CV478840	(GA)10	CAAGGATTAAACCTACGACG	ACTTTCCAATTTCACACCAG	299	55.1
125	CV478859	>gi|53703635|gb|CV478859.1|CV478859	(GCC)4	TCTCTCGATCAAAGATTTCC	ATTCTTAGCCAGGTTCTGGT	330	55.0

Sl.No	Marker name	Sequence ID	Repeat	Left Sequence	Right Sequence	Product Size	Tm
126	CV478864	>gi|53703640|gb|CV478864.1|CV478864	(CCA)4	ACAGGTACCAATCATCAAGC	GTTCTCCGTCATCAACTCAT	383	55.0
127	CV478880	>gi|53703656|gb|CV478880.1|CV478880	(AC)6	CTTCTTCCTCCATCACACAT	AGAGCTACTACCCATTTCCC	130	55.0
128	CV478887	>gi|53703663|gb|CV478887.1|CV478887	(GCG)4	TTCGGGAAGAAAGTAGGAGT	GGAACAGAGAGTCCACCATA	193	55.3
129	CV478888	>gi|53703664|gb|CV478888.1|CV478888	(CAG)7	CAAGCTCAACATGCTACAAA	CCTCCACTCTTCATGTTGTT	221	55.1
130	CV478891	>gi|53703667|gb|CV478891.1|CV478891	(CAA)6	TTCTGATCCTAACTTCACCG	ACAACTCACCGTCTAATCGT	236	55.0
131	CV478896	>gi|53703672|gb|CV478896.1|CV478896	(TGT)4	AGTTCAGATTGCTGAGCTGT	ACAAGGCAACTCACTCACTT	125	55.0
132	CV478902	>gi|53703678|gb|CV478902.1|CV478902	(AAG)4	CGCTTGATAAATGGATCTTC	CTGCCTCCAATTATCTCAAG	376	55.0
133	CV478907	>gi|53703683|gb|CV478907.1|CV478907	(CAG)4	CTTCCCGTTAATGAAGAGTG	ATGATGCTGATGGACTGAAT	217	55.2
134	CV478910	>gi|53703686|gb|CV478910.1|CV478910	(GAA)4	TGGAAGCAAGGTGAATAAGT	CTTCTTCAGGAAGGCTACAA	380	54.9
135	CV478910	>gi|53703686|gb|CV478910.1|CV478910	(GAG)4	TGGAAGCAAGGTGAATAAGT	CTTCTTCAGGAAGGCTACAA	380	54.9
136	CV478913	>gi|53703689|gb|CV478913.1|CV478913	(GAA)6	AAGGGAGTACAAGCTGTTGA	AGTTTACAGACTGCCTTCCA	361	55.0
137	CV478916	>gi|53703692|gb|CV478916.1|CV478916	(AAG)4	ACCAACAGTAATCGAAGACG	TGATACCGGTAGTCTCATCC	299	55.2
138	CV478927	>gi|53703703|gb|CV478927.1|CV478927	(TGA)6	AACTCTTCCTCTTCCACTCC	GTGAAGCTCTGAAACAGGAC	289	55.0
139	CV478933	>gi|53703709|gb|CV478933.1|CV478933	(TCA)4	TAGAAAGCCATATCCCAGAA	GGAGATGTTGTTGTTTCGAT	300	55.0
140	CV478941	>gi|53703717|gb|CV478941.1|CV478941	(AGA)4	CTTCACATCAATCTCCACACT	CACCTGGAAACTTTCATAGC	247	55.0
141	CV478948	>gi|53703724|gb|CV478948.1|CV478948	(AG)9	GGAAAGAGTGTGGGTTGATA	TCTCGTTATTTGTGTTTCCG	199	55.7
142	CV478963	>gi|53703739|gb|CV478963.1|CV478963	(GT)5	CTGACTAATTGCATCTGACG	CGAATTGGTATTCTTAACCG	214	54.7
143	CV478996	>gi|53703772|gb|CV478996.1|CV478996	(ATC)4	TTCTTCCAAATCACCAACTT	TGAAGGTTGAGAGGAAGAGA	112	54.9

Table C.2. List of SSRs, number of repeats and primer information from the Roose-Amsaleg et al. (2006), SSR collection used in the molecular marker study.

Sl.No	Marker name	Sequence ID	Repeat	Left Sequence	Right Sequence	Product Size	Tm
144	LU1	AY849261	(CA)8	GAAGCTCGAACTAGGTGGCC	GGGAGGCAACCCATGTCTAG	241	55.0
145	LU2	AY849262	(TC)18	AACCGGAACTTTCGGCTGAG	GGTTGGAGTAATCGCCGGAG	213	55.0
146	LU3	AY849263	(GT)11	CTTTTTTGAGTCACCAAGCC	CGCTGGAGTCTGAATCCTAG	158	55.0
147	LU4	AY849264	(GA)9	CGAGAACAGTGATTGGCTGC	CCGACCGAACTAGTGGCTTG	167	55.0
148	LU8	AY849268	(AG)24	ACACTTGCTATTAGCTACAAGAGAG	CAGCATCCAGAGGTTCTCAC	216	55.0
149	LU11	AY849271	(TG)7(GT)7	GAGTGCTGCTGGATTCAATG	GATTCCTGCTCCATGACAAA	308	55.0
150	LU12	AY849272	(CA)13	TGAGGATAACGGTAACAACACTGG	GCTTCCACTGGCAAGATCAAT	256	55.0
151	LU13	AY849273	(AC)4(AG)18	TGTGCCAATAGCCATGTGAG	GTATGGCTTCCTATGGGCTAAC	376	55.0
152	LU15	CA483290	(CAT)8	GGGTTATACATTGTTCTTCATTCGG	CAAGAGGAATGCAGGATGCC	208	55.0
153	LU17	AY849275	(GA)26	ATGATCGCATGAGCAAATTG	GTTTGTGAGGTGACGGTGAG	287	55.0
154	LU19	AY849277	(GA)7(GT)9	TATCGTCGCAACTCCATCGG	CCAGCAGCAGCACCAACATG	143	55.0
155	LU20	AY849278	T4(CA)8	AGCAACAATCAACCCTAGCCTG	GGCACTTTTGGTCGATGGAG	214	55.0
156	LU21	AY849279	(GA)15A4	CCGAGTCCGAAAGAATCTGG	CAGCTCCCATTGTTGTTCCC	219	55.0
157	LU23	AY849280	(CA)8(GA)22	CATGACCATGTGATTAGCATCG	CATAGGAGGTGGGTTGCTGC	259	55.0
158	LU27	CA483377	(TC)8	GATCAAACTCCCCCAATCCTC	AGGCGATGGCTCGATCC	176	55.0
159	LU28	CA483374	(TCT)8	TCCCAGCGAGTTTGGTGAG	TGGAGGAACTAATTGTGGCAAG	187	55.0
160	LU29	CA483255	(TC)8	CACTCCTCTTTTCACTTGTCTTCTG	GAAGGTGGTGGTGAGTGCAC	184	55.0
161	LU31	CA483192	(TC)8	CACGCAATCTCTCCCAGACA	GAGAGTTTAGGGTATGCACTGA	138	55.0
162	LU32	CA483178	(AG)10	GAAAAGGAAGGCTTAGAAGAAGAAG	AGTTTCTCAATACACAGATCGAAGG	252	55.0
163	LU35	CA482907	(TCC)8	GATCAAACTCCCTCCAATCCTC	TCTCAGGGTCCACGGTTTG	124	55.0
164	LU36	CA482840	(CT)9	TTCTCTACACACTAGGCAATATCCC	TCAACTGCAATTCCTGGTGAG	190	55.0
165	LU37	CA482834	(TC)8	GTTCATTATCAAACATTTGATCTTATTTG	CCCGTACTTGAGTTAAGTACGTCC	259	55.0
166	LU38	CA482784	(GAA)8	TTACAATACGAAGACTTCGATCCC	TTTGCTTCGGTTAATGGCC	168	55.0

Table C.3. List of SSRs, number of repeats and primer information from the Roach and Deyholos (2007) EST collection submitted in the GenBank used in the molecular marker study.

Sl.No	Marker name	Repeat	Left Sequence	Right Sequence	Product Size	Tm
167	10D10	(GT)11	TTTTGGAATCTGATTCGTTT	TTCACCACCTAAATCCCTAA	100	54.9
168	10G3	(AG)13	GCAAGGAGATGGAAATGTTA	TGCCGTCTCTTCAATAAATAA	100	40.0
169	1C12	(TC)23	CTCTGTCTCTTCAGTCTTCCC	GCTCTAGCAGAAGCATTTTG	100	55.7
170	1F1	(AGAC)6	TCCGGATTGACTCTTCCTTC	AAGATTTCAGACTTTTCAGTGGTTT	102	59.2
171	1F12	(TG)14	GAGGAGATTTGTTGCCAAAGA	ATCTGAAGTTTTCCAGGGCCTTT	100	59.3
172	2B3	(TCA)9	TCTCCATTCTTCTTCCTCAAACA	GGGAGAAGATGGTGGTGATG	108	60.3
173	2D2U	(CT)17	CATCCAACAACTTTTCGAGGA	GCCTCAGATAATCGAAGAATCA	104	55.0
174	2D2L	(CT)17	CATCCAACAACTTTTCGAGGA	GCCTCAGATAATCGAAGAATCA	104	55.0
175	2E4A	(GTA)8	CGATTAATTACCACGACACGA	CCACACAATCAAATTCTAATGGA	115	58.6
176	2E4B	(GACA)7	AGGGTGATGGAAACAGTTTTG	TAGCAGAAGCCTGGAGAAGG	106	58.9
177	2H10	(GA)17	TGAGAGAGTTGAAGGAGGGAAG	CGCTCTAGCAGAAGCCTGAT	102	59.9
178	3A10	(TG)20	TCCTTTGTTTGTCCTCCCTTA	CAAACTCCACACAACCAGCA	102	58.6
179	3A1	(GT)11	TTGGAATTTTGGAATCTGA	CCAACAAGAGCACATGGTCA	126	53.0
180	3B10	(TGTC)7	AAATAAGACTTCAGACTTTTCAGTGG	TTGACTCTTCCTTCCTCCGATG	100	58.5
181	3B5	(GACA)8	CGAGATATATTGGGATGGATCA	TACTGGGGTTTCGTTTCCTG	138	58.7
182	3G11	(GACA)27	CAATTTCAAACTTGGCTCAG	CCGCTCTAGCAGAAGCCTAT	266	56.1
183	3G11O	(GACA)27	TGGCCTAAGTATTATTATCAATTTCG	CCGCTCTAGCAGAAGCCTAT	235	59.0
184	4A3	(GA)15	GTCAAACTATTGCGTCCCGAGT	GGCTTCCAATATGGAGCCTA	100	62.6
185	4C1	(TG)10	CGACGAGTGGAACAAGTGTG	GCCGCTCTAGCAGAAGCAAT	100	60.4
186	4D9	(GACA)7	TCCGGATTGACTCTTCCTTC	GATTTCAGACTTTTCAGTGGTTT	100	59.2
187	4F7	(AGAC)19	TAGGAGGACAAGCAAAAAC	CGGCCGCTCTAGCAGAAG	132	52.9
188	5B6	(GTCT)10	TGAGTGGGTTAGTGGGATCTG	AAGCAGCACGAACCTGTTTT	106	59.9
189	5E 8	(GAT)7	GCCAGCGATGATTACGATGA	CCTAATCAGCACAAATTACATCA	108	56.9

Sl.No	Marker name	Repeat	Left Sequence	Right Sequence	Product Size	Tm
190	5F1	(GA)22	GCGGGTTGGAAAATGAGG	GCCTCTCCCACTCTTTGTCTC	104	60.3
191	6C6	(TC)18	TTTGACCAAGCATACACATACA	AAACAACACAACGCAACAGC	107	56.7
192	6E 7	(ACA)7	CACAGAAATGCCAGAAGAAAAG	CGTTTCTAACATGAGGGCGTTG	100	59.0
193	6F11	(CT)14	GACCATCGTCGTCATTTCCT	AATGCGAAAATCGCCAATAC	115	59.9
194	6H5	(TC)24	TATCCTTGGCATTACGGTCTG	GAAGGCTGTAATTAAGGGTGGT	105	58.5
195	7A2	(GT)18	GCAATTCTTTTGTCCTCAAACA	CCGACGAGCCATGTGAAC	100	59.2
196	7C12	(CTTT)6	TGTTGATAACCTATCTGGTTCAGG	TGGTTGCGTTAACTAACAGAGA	103	57.6
197	7F2	(GACA)8	TTGGGATGGATCAATTCAGTT	CCTTTCATGCAAATGCTTTC	105	58.3
198	8A11	(CAGA)9	ATGAAGCAGCACGAACCTGT	GAGTTGGTTAGTGGGATCTGT	108	55.6
199	8A2	(GTCT)7	TAATAAATAAGATTTCAGACTCTTCAG	CTCTTCCTTCCTCCGACGAT	100	54.1
200	8F9	(TC)20	AACTCCCCTTCATCCTTTCT	TGATAGTATGATTTGGTTGGAAGG	104	56.7
201	8H10	(CA)11	ACGTGGATTAGTAGCCATCTT	CTCTACCTTTCCCCATACAACC	100	55.5
202	9B10	(TC)24	ATCGACCATCGTCGTCATTT	ATCAGCGCTTCGTTTGTCTT	102	60.0
203	9B2	(TG)10	TCTTAAATGGGGGTTTTCGA	CAAACACTTGCTGGTTAGTTGG	101	59.3
204	9F12	(TTC)3	CATGTTGCTTTCATTACAGTTGG	TGCAAATACTTTGAGAAACATTTCAT	104	59.4
205	9F8	(TC)19	AATATGGAGCCCACGCACAG	GAGAGATGAAGGAGGGAAGGA	100	59.7

Table C.4. List of ISSRs and primer information from the Weisner and Weisnerova, (2004) collection used in the flax molecular marker study.

Sl.No	Marker name	Sequence ID	Left Sequence	Right Sequence	Product Size	Tm
206	*lin136*	DQ202353	CTTTCTCACTTCACATGGGACTC	TTGTATGGACGTTGATATTGGATCT	396	60
207	*lin137*	DQ202354	GGTTCAATATGTGTCTAGAAAACACAT	GTTTCCTTGCCACTTTTCCTCATTATT	256	57
208	*lin139*	DQ202355	CACTTGGGTACCTCTGAAATAGAGA	GAATCAAAAGGCTGAAATTATATGG	398	60
209	*lin145*	DQ202356	TTTTTGTTTTGATTTCCTTTCCAT	ATGCATGTCAAATATTAACCGATTT	394	62
210	*lin146*	DQ202357	GAAAGAAGAAGAAAATCGGGAATC	ATTGAAATGAAACTGGAAGAGACTG	400	57
211	*lin148*	DQ202358	GAATGAGTTACAGTTGAAAGGGAAA	TTCTTCTTCTTATTCCCCAAACTCT	402	60
212	*lin602*	DQ202359	CTTTGAGGTTAAGCTGATATTATTGG	TTTCAATATGCAGATAGTCGTTCAG	418	55
213	*lin603*	DQ202360	TCTGAGAGACGACTTATACTCATGC	GGCAAGAAGAGTATTTACTGCACTC	287	60
214	*lin604*	DQ202361	CATAATCTCGATTGTTGCAATAGGT	CAGCATTTGTTCCTGTACCTAAGAT	277	60

Appendix D

Table D.1. Transcript abundance and functional categorization of the remaining probe sets (excluding those in Table 4) in microarray comparisons of stem peels from Viking and E1747.

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
Amino acid and protein metabolism					
1546	4288	18D7	Ubiquitin fusion protein	1.24	Protein metabolism
5407	8623	58C1	Ribosome recycling factor	0.73	Protein biosynthesis
4705	2331	50A8	60S ribosomal protein	0.70	Protein metabolism
18	923	107G8	Elongation factor 1 alpha	0.65	Protein biosynthesis
5063	1090	54B12	40S ribosomal protein	0.65	Protein synthesis
1368	609	16EE12	30S ribosomal protein	0.62	Protein metabolism
974	3751	12A4	Cysteine protease inhibitor	0.61	Catalytic enzyme
107	279	85D8	60S ribosomal protein	0.54	Protein metabolism
1491	9248	17G9	L24 ribosomal protein	0.47	Protein biosynthesis
4402	4874	47F3	60S ribosomal protein	0.45	Protein synthesis
3047	5670	33D10	60S ribosomal protein	0.40	Protein metabolism
2269	4392	26F3	40S ribosomal protein	0.35	Protein metabolism
628	5966	107B10	Lipoprotein A precursor	0.03	Protein biosynthesis
127	7857	87D1	Ribosomal protein	0.03	Protein metabolism
4380	4873	47D3	ATPOB1	-0.43	Protein biosynthesis
125	8912	68C9	Cysteine proteinase precursor	-0.48	Catalytic enzyme
1120	4190	13F7	30S ribosomal protein	-0.52	Protein metabolism

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
1624	9453	19D1	Ngc-D protein	-0.55	Protein binding
1472	4297	17F11	Aminotransferase	-0.64	Protein metabolism
6555	6523	6C10	40S ribosomal protein	-0.65	Protein metabolism
1618	4403	19C3	Cysteine protease	-0.72	Catalytic enzyme
4539	9889	49B9	Cdc2MsC like protein kinase	-0.85	Protein biosynthesis
5172	5498	55D7	60S ribosomal protein L12	-0.85	Protein metabolism
4673	997	4F8	Alpha-1,4-glucan phosphorylase	-0.92	Protein metabolism
801	1754	10B4	Initiation factor 3g	-1.17	Protein biosynthesis
4654	996	4D8	Ubiquitin conjugating enzyme	-1.33	Protein metabolism
Defense					
259	8469	86EE8	DnaK protein	2.69	Defense
2851	4567	31B11	Heat shock protein	0.93	Defense
4914	8570	52D5	Heat shock protein	0.78	Defense
2168	5547	24EE10	Leucine-rich protein	0.57	Defense
7364	269	78B4	GDSL-motif lipase	0.48	Defense
114	1353	78G4	major latex-like protein	0.38	Defense
198	8993	87C8	PR protein	-0.01	Defense
208	9365	6F12	major latex protein	-0.18	Defense
238	8286	4H3	PR protein	-0.39	Defense
96	2791	78F12	blight- p12 precursor	-0.43	Defense
95	727	36A4	Heat shock factor	-0.49	Defense

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
3035	8203	33C1	major latex-like protein	-0.54	Defense
2881	4572	31D7	Uridylyl-transferase	-0.61	Defense
23	6017	4EE7	Cysteine proteinase	-0.66	Defense
Neucleotide biosynthesis					
4342	9781	46H9	Dihydropyrimidinase	1.11	DNA binding protein
8291	7961	90F9	Histone	0.60	DNA binding protein
1220	1806	14G4	Glutaredoxin	0.55	Signal/DNA synthesis
1399	1826	16G8	DnaJ protein	-0.36	DNA binding protein
113	3770	55C3	DnaJ-like protein	-0.44	DNA binding protein
4551	9840	49C9	DnaJ-related chaperone protein	-0.58	DNA binding protein
4983	1181	53B8	SWIB complex protein	-0.73	DNA binding protein
93	4505	15EE12	RNA polymerases	-0.87	DNA binding protein
4715	2381	50B8	Methyl-CpG-DNA binding domain	-0.98	DNA metabolism
5945	6404	63C6	Putative splicing factor	-1.03	DNA metabolism
Other biosynthesis					
124	977	105F4	SAH7	0.89	Pollen allergen
8084	5471	89D6	SAH7	0.68	Pollen allergen
88	5680	43EE4	Senescence-associated protein	0.67	Other protein
1063	4138	13A7	fiddlehead-like protein	-0.33	Other protein
8908	3460	98F7	Phenylalanine ammonia lyase	-0.48	Alkaloid biosynthesis

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
8475	350	93G12	Uroporphyrin-III C-methyltransferase	-0.84	Porphyrin biosynthesis
4543	2340	49C12	Peroxidase POA1	-0.98	Secondary metabolism
6015	6362	64B10	UDP-glucose glucosyltransferase	-1.25	Flavonoid biosynthesis
Photosynthesis					
130	2983	73F12	Chlorophyll binding protein	-0.25	Photosynthesis
739	9235	6G8	Chlorophyll binding protein	-0.32	Photosynthesis
185	3062	94B7	Photosystem I subunit XI	-0.39	Photosynthesis
2487	9464	28B1	Class III HD-Zip protein 3	-0.41	Photosynthesis
2638	4450	75F11	Photosystem I subunit XI	-0.46	Photosynthesis
1848	9321	19EE9	Chlorophyll binding protein	-0.50	Photosynthesis
4371	7319	47C6	PAR-1b-like protein	-0.52	Photosynthesis
1799	3071	20D11	Zeaxanthin epoxidase	-0.53	Photosynthesis
Primary energy metabolism					
488	8393	105D9	Cyclophylin	0.86	Catalytic enzyme
5691	6268	60D6	Aconitate hydratase	0.67	Energy
8105	383	89F4	Cyclophylin	0.6	Catalytic enzyme
5928	1374	63B12	Serine hydroxymethyltransferase	0.45	One carbon pathway
182	1531	69A4	Aspartate aminotransferase	0.3	Energy
7404	7771	78F1	Fructose-bisphosphate aldolase	-0.51	Catalytic enzyme
3859	7226	41EE2	Thylakoid membrane phosphoprotein	-0.54	Energy

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
1212	6851	14F6	NAD(+) pyrophosphorylase	-0.61	Glycometabolisim
7561	9015	7C5	Chlorophyll binding protein	-0.82	Energy
Signaling and gene regulation					
8244	2963	90B7	Receptor like protein kinase	1.50	Signal transduction
7460	2753	79B7	Receptor like protein kinase	1.25	Signal transduction
145	8270	71F9	Tonoplast intrinsic protein	0.74	Translation
4269	7278	46B10	MYST1	0.74	Transcription
5331	8640	57C9	14-3-3 protein	0.60	Signal transduction
193	283	85D4	Translation elongation factor	0.51	Translation
138	530	97C4	Gibberellin regulated protein	0.49	Signal transduction
3749	7105	40C10	Calmodulin	-0.50	Signal transduction
5701	8719	60EE5	Sm ribonucleoprotein	-0.51	Transcription
4907	8574	52D1	Armadillo protein	-0.60	Signal transduction
280	438	102A8	YDA kinase	-0.63	Cell cycle
8769	496	97B12	Translation initiation factor	-0.64	Translation
51	1431	65G8	Myb domain protein 59	-0.66	Transcription
64	9268	15F1	Transcription factor	-0.67	Transcription
1334	5657	16B10	Anaphase promoting complex	-0.74	Cell cycle
7333	132	77G8	ATSWI3C	-0.82	Transcription
8427	2848	93C11	Geranyl-geranyl-transferase	-0.92	Signal transduction

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
Unknown					
102	9322	19G9	no blastx hit at E<0.0000000001	3.10	unknown
1889	9362	21D5	no blastx hit at E<0.0000000001	1.87	unknown
2862	2969	90F3	no blastx hit at E<0.0000000001	1.77	unknown
4100	9753	44B9	no blastx hit at E<0.0000000001	1.75	unknown
1029	6777	12F6	no blastx hit at E<0.0000000001	1.42	unknown
7712	2649	85A11	no blastx hit at E<0.0000000001	1.39	unknown
5844	8792	62B5	unnamed protein product	1.18	unknown
2003	3328	73EE3	no blastx hit at E<0.0000000001	1.15	unknown
4313	2280	46F12	no blastx hit at E<0.0000000001	1.13	unknown
3581	5892	39D2	no blastx hit at E<0.0000000001	1.11	unknown
7179	2687	76B11	unnamed protein product	1.10	unknown
7654	241	80C8	no blastx hit at E<0.0000000001	1.07	unknown
291	7943	102C1	no blastx hit at E<0.0000000001	1.06	unknown
4401	2366	47F12	no blastx hit at E<0.0000000001	1.02	unknown
2004	3192	22F3	unnamed protein product	1.00	unknown
8375	2957	91F3	unnamed protein product	0.97	unknown
1373	5613	16EE6	no blastx hit at E<0.0000000001	0.93	unknown
4531	4889	49B11	no blastx hit at E<0.0000000001	0.92	unknown
5755	8879	61B5	no blastx hit at E<0.0000000001	0.85	unknown
132	3123	33A7	no blastx hit at E<0.0000000001	0.84	unknown

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
4245	9743	45G9	unnamed protein product	0.82	unknown
1843	573	20H12	no blastx hit at E<0.0000000001	0.80	unknown
8669	7974	95H9	no blastx hit at E<0.0000000001	0.79	unknown
4276	4782	46B7	no blastx hit at E<0.0000000001	0.77	unknown
6272	6506	67A2	no blastx hit at E<0.0000000001	0.77	unknown
3233	7044	35EE10	no blastx hit at E<0.0000000001	0.76	unknown
4945	8522	52G5	no blastx hit at E<0.0000000001	0.73	unknown
20	7364	47B10	no blastx hit at E<0.0000000001	0.73	unknown
40	339	88A8	no blastx hit at E<0.0000000001	0.72	unknown
4362	4868	47B7	unnamed protein product	0.72	unknown
5170	1260	55D4	no blastx hit at E<0.0000000001	0.72	unknown
136	7188	42D2	unnamed protein product	0.72	unknown
8316	5322	91A10	unnamed protein product	0.71	unknown
1768	3103	20A3	no blastx hit at E<0.0000000001	0.71	unknown
3	2009	32A8	no blastx hit at E<0.0000000001	0.71	unknown
6334	1554	67F8	no blastx hit at E<0.0000000001	0.70	unknown
777	3581	91G7	unnamed protein product	0.68	unknown
1539	6784	18D10	no blastx hit at E<0.0000000001	0.67	unknown
3595	8311	39EE5	no blastx hit at E<0.0000000001	0.66	unknown
1760	5070	1H6	no blastx hit at E<0.0000000001	0.66	unknown
1489	3896	66D3	no blastx hit at E<0.0000000001	0.65	unknown

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
543	6053	106B10	unnamed protein product	0.65	unknown
5903	1245	62G8	unnamed protein product	0.64	unknown
314	436	102EE12	no blastx hit at E<0.0000000001	0.64	unknown
5071	6041	54C10	no blastx hit at E<0.0000000001	0.63	unknown
6139	1426	65EE12	no blastx hit at E<0.0000000001	0.63	unknown
14	2137	42A4	no blastx hit at E<0.0000000001	0.63	unknown
4764	4838	50G3	unnamed protein product	0.62	unknown
4799	3660	51B3	no blastx hit at E<0.0000000001	0.62	unknown
3518	2172	38F8	no blastx hit at E<0.0000000001	0.60	unknown
8234	7909	90A9	no blastx hit at E<0.0000000001	0.59	unknown
1642	6825	19EE6	no blastx hit at E<0.0000000001	0.57	unknown
964	6666	11H6	no blastx hit at E<0.0000000001	0.56	unknown
3103	3231	34A11	no blastx hit at E<0.0000000001	0.55	unknown
46	322	91A12	no blastx hit at E<0.0000000001	0.52	unknown
2115	1473	94F12	no blastx hit at E<0.0000000001	0.51	unknown
719	6078	108B10	unnamed protein product	0.50	unknown
4555	2390	49D12	no blastx hit at E<0.0000000001	0.49	unknown
5022	6598	53F12	no blastx hit at E<0.0000000001	0.47	unknown
252	1265	60F12	no blastx hit at E<0.0000000001	0.46	unknown
4147	7206	44G10	no blastx hit at E<0.0000000001	0.45	unknown
4	3433	78C12	no blastx hit at E<0.0000000001	0.45	unknown

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
5452	3617	58G11	no blastx hit at E<0.0000000001	0.45	unknown
4449	3987	48B8	unnamed protein product	0.45	unknown
178	6648	60A3	no blastx hit at E<0.0000000001	0.41	unknown
8401	2954	91H7	no blastx hit at E<0.0000000001	0.39	unknown
6383	6420	68C2	unnamed protein product	0.17	unknown
4688	3494	91F7	no blastx hit at E<0.0000000001	0.02	unknown
2060	1928	3F4	no blastx hit at E<0.0000000001	-0.08	unknown
7	7325	74H12	no blastx hit at E<0.0000000001	-0.11	unknown
21	4037	70EE11	no blastx hit at E<0.0000000001	-0.19	unknown
1637	7239	19EE11	no blastx hit at E<0.0000000001	-0.21	unknown
220	3155	20F3	no blastx hit at E<0.0000000001	-0.31	unknown
725	6082	108B6	no blastx hit at E<0.0000000001	-0.36	unknown
2274	9384	25F9	no blastx hit at E<0.0000000001	-0.40	unknown
212	914	98EE4	no blastx hit at E<0.0000000001	-0.44	unknown
118	6121	94C7	no blastx hit at E<0.0000000001	-0.45	unknown
555	1004	106C12	no blastx hit at E<0.0000000001	-0.47	unknown
6714	750	71A8	unnamed protein product	-0.48	unknown
35	3989	70H11	no blastx hit at E<0.0000000001	-0.48	unknown
128	8174	106F12	no blastx hit at E<0.0000000001	-0.50	unknown
6394	6470	68D2	no blastx hit at E<0.0000000001	-0.51	unknown
1260	323	91C12	unnamed protein product	-0.51	unknown

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
6279	8923	67A9	unnamed protein product	-0.51	unknown
736	3533	108C7	no blastx hit at E<0.0000000001	-0.51	unknown
6917	3248	73C7	no blastx hit at E<0.0000000001	-0.52	unknown
4969	1131	53A8	no blastx hit at E<0.0000000001	-0.52	unknown
5175	8711	55EE1	unnamed protein product	-0.52	unknown
8922	3457	98H11	no blastx hit at E<0.0000000001	-0.52	unknown
5618	78	5F12	no blastx hit at E<0.0000000001	-0.53	unknown
3087	5701	33G6	no blastx hit at E<0.0000000001	-0.54	unknown
738	6079	108D10	no blastx hit at E<0.0000000001	-0.54	unknown
22	1196	57H8	no blastx hit at E<0.0000000001	-0.55	unknown
407	935	103EE8	unnamed protein product	-0.55	unknown
1612	1873	19B8	no blastx hit at E<0.0000000001	-0.55	unknown
4747	4833	50EE7	unnamed protein product	-0.55	unknown
4924	6021	52EE6	no blastx hit at E<0.0000000001	-0.55	unknown
7313	2677	77F11	no blastx hit at E<0.0000000001	-0.55	unknown
8597	471	95B12	no blastx hit at E<0.0000000001	-0.56	unknown
727	1082	108B8	unnamed protein product	-0.57	unknown
5556	1357	59H8	no blastx hit at E<0.0000000001	-0.57	unknown
1998	8134	22EE9	unnamed protein product	-0.57	unknown
67	8005	95C1	no blastx hit at E<0.0000000001	-0.58	unknown
4895	8524	52C1	no blastx hit at E<0.0000000001	-0.58	unknown

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
1771	5524	20A6	unnamed protein product	-0.59	unknown
3001	2012	32G8	unnamed protein product	-0.59	unknown
753	1034	108EE8	no blastx hit at E<0.0000000001	-0.60	unknown
8997	3373	99F7	no blastx hit at E<0.0000000001	-0.60	unknown
5397	6164	58B10	no blastx hit at E<0.0000000001	-0.60	unknown
3207	7096	35B6	no blastx hit at E<0.0000000001	-0.62	unknown
4634	3442	4C11	no blastx hit at E<0.0000000001	-0.63	unknown
6710	3301	71A3	no blastx hit at E<0.0000000001	-0.63	unknown
54	1962	28F8	no blastx hit at E<0.0000000001	-0.63	unknown
3175	5738	34G6	no blastx hit at E<0.0000000001	-0.64	unknown
7556	4011	7C11	unnamed protein product	-0.65	unknown
790	4204	10A3	no blastx hit at E<0.0000000001	-0.66	unknown
7238	2648	76G3	unnamed protein product	-0.66	unknown
5164	8702	55C9	unnamed protein product	-0.67	unknown
148	5912	98A2	unnamed protein product	-0.67	unknown
5091	8550	54EE1	no blastx hit at E<0.0000000001	-0.67	unknown
734	3537	108C3	no blastx hit at E<0.0000000001	-0.68	unknown
6413	3963	68F11	no blastx hit at E<0.0000000001	-0.68	unknown
4523	2347	49A4	unnamed protein product	-0.70	unknown
5080	8541	54C9	no blastx hit at E<0.0000000001	-0.70	unknown
8493	479	93H8	no blastx hit at E<0.0000000001	-0.70	unknown

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
8689	8042	96C1	no blastx hit at E<0.0000000001	-0.71	unknown
5435	8620	58EE5	no blastx hit at E<0.0000000001	-0.71	unknown
7207	7692	76D5	unnamed protein product	-0.71	unknown
4660	3526	4EE3	no blastx hit at E<0.0000000001	-0.72	unknown
4702	2335	50A4	unnamed protein product	-0.74	unknown
7425	2718	78G7	no blastx hit at E<0.0000000001	-0.75	unknown
7856	2817	86G7	no blastx hit at E<0.0000000001	-0.76	unknown
7405	5263	78F10	no blastx hit at E<0.0000000001	-0.76	unknown
4138	5125	79C10	no blastx hit at E<0.0000000001	-0.78	unknown
3116	5789	34B2	no blastx hit at E<0.0000000001	-0.79	unknown
6364	3919	68A3	no blastx hit at E<0.0000000001	-0.79	unknown
103	232	85A4	no blastx hit at E<0.0000000001	-0.79	unknown
3290	5777	36B2	no blastx hit at E<0.0000000001	-0.80	unknown
5468	1171	58H8	no blastx hit at E<0.0000000001	-0.81	unknown
6646	1490	70B8	no blastx hit at E<0.0000000001	-0.82	unknown
8384	325	91G12	no blastx hit at E<0.0000000001	-0.82	unknown
8682	583	96B12	no blastx hit at E<0.0000000001	-0.82	unknown
2958	9564	32D1	unnamed protein product	-0.82	unknown
6001	1456	63H8	no blastx hit at E<0.0000000001	-0.82	unknown
4917	8566	52D9	no blastx hit at E<0.0000000001	-0.83	unknown
5654	6221	60A2	unnamed protein product	-0.83	unknown

Probe set #	UID #	Glycerol stock #	Probe set annotation	Fold enrichment (Viking/ E1747)	Functions
2855	9571	31B5	no blastx hit at E<0.0000000001	-0.84	unknown
3154	737	34EE8	no blastx hit at E<0.0000000001	-0.84	unknown
5519	1310	59EE4	unnamed protein product	-0.86	unknown
3607	8361	39F5	unknown [Populus trichocarpa]	-0.87	unknown
1479	1876	17F8	unnamed protein product	-0.94	unknown
6669	6438	70EE10	unnamed protein product	-0.94	unknown
2602	4494	29D11	no blastx hit at E<0.0000000001	-0.95	unknown
3433	2086	37F8	unknown [Populus trichocarpa]	-0.99	unknown
268	7778	85B5	no blastx hit at E<0.0000000001	-0.99	unknown
1829	3151	20F7	no blastx hit at E<0.0000000001	-1.05	unknown
7242	144	76G8	no blastx hit at E<0.0000000001	-1.12	unknown
1255	9262	15B5	unnamed protein product	-1.13	unknown
1504	1088	108F4	no blastx hit at E<0.0000000001	-1.26	unknown
2791	4486	30D7	no blastx hit at E<0.0000000001	-1.27	unknown
5319	3693	57B7	no blastx hit at E<0.0000000001	-1.42	unknown
2175	5358	87F2	no blastx hit at E<0.0000000001	-1.49	unknown
1527	3021	20C11	no blastx hit at E<0.0000000001	-1.61	unknown

Table D.2. Primer sequence information for the quantitative real time PCR analysis.

Sl.No.	Transcript	Primer name	Sequence
1	Peroxidase precursor	57B6_PERO-F	5' gcg cct aca tct tca ctt aac ga 3'
		57B6_PERO-R	5' atg gtg tgg gat ccg gat ag 3'
2	Fasciclin-like AGP 4	c262_FLA9_F	5' cgt ccg cgt caa tat tag ca 3'
		c262_FLA9_F	5' cct tgt cca cct ggt aaa tcg 3'
3	Ubiquitin conjugating enzyme-like	4D8_UBI-F	5' tct ctc ggt gtt gaa gaa aag gt 3'
		4D8_UBI-R	5' tcg tcc cca cca act gaa tc 3'
4	N-hydroxylating cytochrome P450	52F6_Ncyt_F	5' aat ggc gga gct gat aaa aca 3'
		52F6_Ncyt_R	5' cgg att ctt gga cta gcc tat ctt 3'
5	Lipid transfer protein 4 precursor	LuLTPA_427F	5' gaa gtg aga ccg acc gaa tga 3'
		LuLTPA_526R	5' acg tag cga cgt cac aaa agt c 3'
6	β - galactosidase	LuGAL_253F	5' aac tgc ggt ggc tgt tct tac 3'
		LuGAL_352R	5' atg acc gcg gaa cat ggt a 3'